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RECOMBINANT DNA RESEARCH
Volume 6

Documents Relating to
"NIH Guidelines for Research
Involving Recombinant DNA Molecules"
January 1980 - December 1980

April 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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RECOMBINANT DNA RESEARCH
Volume 6

Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules"
January 1980 - December 1980

April 1981

Prepared by the
Office of the Director, NIH

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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PREFACE

This document is the sixth in a series constituting a "public record" of activities in regard to the NIH Guidelines for recombinant DNA research.

The first five volumes cover events from February 1975 through January 1980.

This sixth volume covers events from January 1980 through December 1980 culminating in the revised "NIH Guidelines for Research Involving Recombinant DNA Molecules" as published in the Federal Register on November 21, 1980. These appear first in this volume.

The other volumes in this series may be purchased from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, or viewed in some 600 public libraries of the GPO depository system. The GPO stock number of Volume 1 is 017-040-00398-6; Volume 2, 017-040-00422-2 and (supplement: Environmental Impact Statement) 017-040-00413-3; Volume 3, 017-040-00429-0 and (appendices) 017-040-00430-3; Volume 4, 017-040-00443-5 and (appendices) 017-040-00442-7; and Volume 5, 017-040-00470-2.

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federal register

Friday
November 21, 1980

Part VII

Department of Health and Human Services

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules, November 1980

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20205

December 3, 1980

TO: Recipients of Guidelines for Research Involving Recombinant DNA Molecules

FROM: Office of Recombinant DNA Activities, NIH

SUBJECT: Effective Date of the Revised Guidelines

The Federal Register inserted an effective date of November 21, 1981 by mistake on page 77372 of the Federal Register of November 21, 1980.

The correct effective date of the revised Guidelines is:

NOVEMBER 21, 1980

HEALTH AND HUMAN SERVICES DEPARTMENT

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules, November 1980

These NIH Guidelines supersede those of January 1980, and will be in effect until further notice.

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I. Scope of the Guidelines

I–A. *Purpose.* The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

I–B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the republication of those described in (i) above.

I–C. *General Applicability.* See Section IV–B.

I–D. *Prohibitions.* The following experiments are not to be initiated at the present time:

I–D–1. Formation of recombinant DNAs derived from the pathogenic organisms classified (*1*) as Class 4 or 5 (*2*) or from cells known (*2A*) to be infected with such agents, regardless of the host-vector system used.

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (*2A*) (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.).

I–D–3. (Deleted).

I–D–4. Deliberate release into the environment of any organism containing recombinant DNA.

I–D–5. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. (*2A*)

I–D–6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established (*a*). (See Section IV–E–1–b–(3)–(d).)

I–D (1–6). Experiments in Categories I–D–1 to I–D–6 may be expected (*4*) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, National Institutes of Health (NIH), with advice of the Recombinant DNA Advisory Committee (RAC), after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(e).)

Experiments in Categories I–D–1, I–D–2, I–D–5, and experiments involving "wild type" host-vector systems are excepted from the prohibitions, provided that these experiments are designed for risk-assessment purposes and are conducted within the NIH high-containment facilities located in Building 41–T on the Bethesda campus and in Building 550 located at the Frederick Cancer Research Center. The selection of laboratory practices and containment equipment for such experiments shall be approved by the Office of Recombinant DNA Activities (ORDA) following consultation with the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. ORDA shall inform RAC members of the proposed risk-assessment projects at the same time it seeks consultation from the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. If a major biohazard is determined, the clones will be destroyed after the completion of the experiment rather than retaining them in the high containment facility. Other clones that are non-hazardous or not of major hazard will be retained in the high containment.

I–E. *Exemptions.* It must be emphasized that the following exemptions (*4*) are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms (*1*) or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5.

The following recombinant DNA molecules are exempt from these Guidelines, and no registration with NIH is necessary:

I–E–1. Those that are not in organisms or viruses. (*5*)

I–E–2. Those that consist entriely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

I–E–3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

I–E–4. Ceratin specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

I–E–5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland, 20205.

I–F. *General Definitions.* See section IV–C.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information, therefore, already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. (*6–19*) The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs, by their very nature, lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a *vector*, or *vehicle*, (plasmid or virus) for specific hosts or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the Guidelines.

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and institutuional biosafety committees recommend changes in the Guidelines to permit their use.

II–A. *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. (*6–15*) Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. (see Sections IV–D–1–g, IV–D–5–d and IV–D–6–b.). This shall, as a minimum, include instructions in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (See Sections IV-D-5-e and IV-D-3-d.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provide. (See Sections IV-D-1-h and IV-D-8-c.)

II-B. *Physical Containment Levels.* The objective of physical containment is to confine organisms containing recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. For example, the "Classification of Etiologic Agents on the Basis of Hazard,"(*7*) prepared by the Centers for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels.(*8*)

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV-E-1-b-(2)-(b).) Additional material on physical containment for plant host-vector systems is found in Sections III-C-3 and III-C-4.

II-B-1. *P1 Level.*

II-B-1-a. *Laboratory Practices.*

II-B-1-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-1-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-1-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decomtaminated before washing, reuse, or disposal.

II-B-1-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-1-a-(5). Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-1-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-1-a-(7). Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

II-B-1-a-(8). Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-1-a-(9). An insect and rodent control program shall be instituted.

II-B-1-a-(10). The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

II-B-1-a-(11). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-1-a-(12). The laboratory shall be kept neat and clean.

II-B-1-b. *Containment Equipment.* Special containment equipment is not required at the P1 level.

II-B-1-c. *Special Laboratory design.* Special laboratory design is not required at the P1 level.

II-B-2. *P2 Level.*

II-B-2-a. *Laboratory Practices.*

II-B-2-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-2-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-2-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

II-B-2-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-2-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-2-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-2-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-2-a-(8). Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-2-a-(9). Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

II-B-2-a-(10). The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-2-a-(11). An insect and rodent control program shall be instituted.

II-B-2-a-(12). The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II-B-2-a-(13). Animals not related to the experiment shall not be permitted in the laboratory.

II-B-2-a-(14). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-2-a-(15). The laboratory shall be kept neat and clean.

II-B-2-a-(16). Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II-B-2-b. *Containment Equipment.* Biological safety cabinets (20) shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

II-B-2-c. *Special Laboratory Design.* An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

II-B-3. *P3 Level.*

II-B-3-a. *Laboratory Practices.*

II-B-3-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-3-a-(2). Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-3-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II-B-3-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-3-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-3-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-3-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-3-a-(8). Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-3-a-(9). Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

II-B-3-a-(10). Persons under 16 years of age shall not enter the laboratory.

II-B-3-a-(11). The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-3-a-(12). An insect and rodent control program shall be instituted.

II-B-3-a-(13). Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

II-B-3-a-(14). Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

II-B-3-a-(15). Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

II-B-3-a-(16). Animals and plants not related to the experiment shall not be permitted in the laboratory.

II-B-3-a-(17). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II-B-3-a-(18). Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-3-a-(19). The laboratory shall be kept neat and clean.

II-B-3-a-(20). If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

II-B-3-b. *Containment Equipment.*

II-B-3-b-(1) Biological safety cabinets (*20*) shall be used for all equipment and manipulations that produce aerosols—e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging—where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

II-B-3-b-(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units (19A), open cages placed in ventilated enclosures, solid-wall and bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors. (Note: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

II-B-3-b-(3). *Alternative Selection of Containment Equipment.*

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

Table I.—*Combinations of Containment Safeguards*

Classification of experiment According to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological[1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV3	P3	P3	P3	HV3
P3	HV3	P3	P3	P4	HV2
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P2	HV3

Table I.—*Combinations of Containment Safeguards*

Classification of experiment According to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological[1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P3	P2	HV2

[1] See Section II-D for decription of biological containment.

II–B–3–c. *Special Laboratory Design.*

II–B–3–c–(1). The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

II–B–3–c–(2). The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

II–B–3–c–(3). A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near each primary laboratory exit area.

II–B–3–c–(4). Windows in the laboratory shall be sealed.

II–B–3–c–(5). An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II–B–3–c–(6). The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential (i.e., from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

II–B–3–c–(7). The treated exhaust-air from Class I and Class II biological safety cabinets [*20*]may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet and the building ventilation system.

II–B–4. *P4 Level.*

II–B–4–a. *Laboratory Practices.*

II–B–4–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–4–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

II–B–4–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–4–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–4–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

II–B–4–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–4–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–4–a–(8). Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

II–B–4–a–(9). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

II–B–4–a–(10). Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

II–B–4–a–(11). Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

II–B–4–a–(12). Persons under 18 years of age shall not enter the P4 facility.

II–B–4–a–(13). Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

II–B–4–a–(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

II–B–4–a–(15). the universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

II–B–4–a–(16). An insect and rodent control program shall be instituted.

II–B–4–a–(17). Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

II-B-4-a-(18). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II-B-4-a-(19). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II-B-4-a-(20). The laboratory shall be kept neat and clean.

II-B-4-a-(21) If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4-level containment, they shall be conducted in accordance with all P4-level laboratory practices specified in this section.

II-B-4-b. *Containment Equipment.*

II-B-4-b-(1). Experimental procedures involvong organisms that require P4-level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure isolation suits.

II-B-4-b-(2). Laboratory animals involved in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units [19A], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, or solid-well and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure suits.

II-B-4-b-(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II

Table II.—*Combinations of Containment Safeguards*

Classification of experiment according to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			Biological containment
Physical containment	Biological [1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	[2] P4	P3	HV2

[1] See Section II-D for description of biological containment.

[2] In this case gloves shall be worn, in addition to the clothing requirements specified in II-B-4-a-(14).

II-B-4-c. *Special Laboratory Design.*

II-B-4-c-(1). The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

II-B-4-c-(2). Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces ar sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

II-B-4-c-(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

II-B-4-c-(4). Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply, liquid and gaseous services provided to the P4 facility shall be protected by devices that prevent backflow.)

II-B-4-c-(5). Drinking water fountains shall not be installed in laboratory or animal rooms of the P4 facility. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

II-B-4-c-(6). Laboratory doors shall be self-closing.

II-B-4-c-(7). A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

II-B-4-c-(8). A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

II-B-4-c-(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be activated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

II-B-4-c-(10). An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

II-B-4-c-(11). Air within individual laboratories of the P4 facility may be recirculated if HEPA filtered.

II-B-4-c-(12). The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow *in situ* decontamination before removal and to facilitate certification testing after replacement.

II-B-4-c-(13). The treated exhaust-air from Class I and Class II biological safety cabinets(*20*) may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets of the facility exhaust air system.

II–B–4–c–(14). As noted in Section II–B–4–b–(1), the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be airtight. The exhaust-air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

II–C. *Shipment.* Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation (§ 72.25, Part 72, Title 42, and §§ 173.386–.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR)) as specified below:

II–C–1. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of § 72.25(c)(1—(5), Part 72, Title 42 CFR, and §§ 173.386–.388, Part 173, Title 49 CFR.

II–C–2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under II–C–1 and are also subject to § 72.25(c)(6), Part 72, Title 42 CFR.

II–C–3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research."

II–D. *Biological Containment.*

II–D–1. *Levels of Biological Containment.* In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

II–D–1–a. *HV1.* A host-vector system which provides a moderate level of containment. *Specific systems:*

II–D–1–a–(1). *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1, or derivatives thereof [*21–27*]) and variants of bacteriophage, such as lambda (*28–33*). The *E. coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages, except as specified in Section III–0.

II–D–1–a–(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Section II—D–2).

II–D–1–b. *HV2.* These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. *Specific systems:*

II–D–1–b–(1). For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

II–D–1–b–(2). For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

II–D–1–c. *HV3.* These are host-vector systems in which:

II–D–1–c–(1). All HV2 criteria are met.

II–D–1–c–(2). The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

II–D–1–c–(3). No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or on unique laboratory-controlled conditions or is blocked by the inserted DNA.

II–D–1–c–(4). The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

II–D–1–c–(5). The relevant genotypic and phenotypic traits have been independently confirmed.

II–D–2. *Certification of Host-Vector Systems.*

II–D–2–a. *Responsibility.* HV1 systems other than *E. coli* K–12, and HV2 and HV3 host-vector systems, may not be designated as such until they have been certified by the director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

Host-vector systems that are proposed for certification will be reviewed by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). (See Section IV–E–1–b–(1)–(c).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available information at a regular meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH.

without review by the RAC. (See Section IV–E–1–b–(3)–(f).)

When new host-vector systems are certified, notice of the certification will be sent by the Office of Recombinant DNA Activities (ORDA) to the applicant and to all Institutional Biosafety Committees (IBCs) and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system. (See Section IV–E–1–b–(3)–(i).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the Director, NIH. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

II–D–2–b. *Data To Be Submitted for Certification.*

II–D–2–b–(1). *HV1 Systems Other than E. Coli K–12.* The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

II–D–2–b–(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted (*32A, 33B*). In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under nonpermissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage λ in *E. coli* K–12 are available from ORDA.

II–D–2–b–(3). *HV3 Systems.* Putative HV3 systems must, as the first step in certification, be certified as HV2 systems. Systems which meet the criteria given above under II–D–1–(c)–1, II–D–1–(c)–2, and II–D–1–(c)–3 will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selected by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated. Tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

II–D–3. *Distribution of Certified Host-Vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates. If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotypic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH; and (iv) remind the user of responsibility for notifying ORDA of any discrepancies with the reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Containment guidelines for permissible experiments are given in Part III. For these experiments no registration with the National Institutes of Health (NIH) is necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene in the cloning vehicle and if so, what protein, and (e) the containment conditions specified by these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals: IBC review prior to initiation of the experiment is not required for most experiments described in Section III–O. Prior IBC review is required for all other experiments described in the subsections of Part III.

Changes from the levels specified in Part III for specific experiments (or the assignment of levels to experiments not explicitly considered here) may not be instituted without the express approval of the Director, NIH. (See Sections IV–E–1–b–(1)–(a), IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

In the classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimal for the experiments designated. The use of higher levels of biological containment (HV3<HV2<HV1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems and prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Experiments using *E. coli* K–12 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–E–1–b–2–(e)).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host-vector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used]. For these experiments review by the IBC prior to the initiation of the experiment is not required. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information (*35*) with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

III–O–1. *Experiments Involving Class 3 Organisms.* Experiments involving recombinant DNA from Class 3 organisms (*1*) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section (IV–E–1–b–2–(e)).

III–A. *Classification of Experiments Using Certain HV1 and HV2 Host-Vector Systems.* Certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of this Section III–A. Those so classified as of publication of these revised Guidelines are listed in Appendix D. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

III–A–1. *Shotgun Experiments.* These experiments involve the production of recombinant DNAs between the vector and portions of the specified cellular source, preferably a partially purified fraction. Care should be taken either to preclude or eliminate contaminating microoganisms before isolating the DNA.

III–A–1–a. *Eukaryotic DNA Recombinants.*

III–A–1–a–(1). *Primates.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III–A–1–a–(2). *Other Mammals.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III–A–1–a–(3). *Birds.* P2 physical containment + an HV2 host-vector, or P3 + HV1.

III–A–1–a–(4). *Cold-Blooded Vertebrates.* P2 physical containment + an HV1 host-vector or P1 + HV2. If the eukaryote is known to produce a potent polypeptide toxin, (*34*) the containment shall be increased to P3 + HV2.

III–A–1–a–(5). *Other Cold-Blooded Animals and Lower Eukaryotes.* This large class of eukaryotes is divided into two groups:

III–A–1–a–(5)–a. Species that are known to produce a potent polypeptide toxin (*34*) that acts in vertebrates, or are known pathogens listed in Class 2, (*1*) or are known to carry such pathogens must use P3 physical containment + an HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3+HV1 or P2+HV2. Species that produce potent toxins that affect invertebrates or plants but not vertebrates require P2+HV2 or P3+HV1. Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group, unless the organisms used as the source of DNA have been shown not to contain those agents, in which case they may be placed in the following group. (*2A*)

III–A–1–a–(5)–(b). The remainder of the species in this class including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2+HV1 or P1+HV2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of disease-causing microorganisms or must belong to a species that does not carry microorganisms causing disease in vertebrates or plants. (*2A*) If these conditions cannot be met, experiments must be done under P3+HV1 or P2+HV2 containment.

III–A–1–a–(6). *Plants.* P2 physical containment + an HV1 host-vector, or P1+HV2. If the plant source makes a potent polypeptide toxin, (*34*) the containment must be raised to P3 physical containment + HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked plant genes, containment may be reduced to P3+HV1 or P2+HV2. (*2A*)

III–A–1–b. *Prokaryotic DNA Recombinants.* P2+HV1 or P1+HV2 for experiments with phages, plasmids and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. (*34*) P3+HV2 for experiments with phages, plasmids and DNA from Class 2 agents. (*1*)

III–A–2–a. *Viruses of Eukaryotes* (summary given in Table III; see also exception given at asterisk at end of Appendix D).

III–A–2–a–(1). *DNA Viruses.*

III–A–2–a–(1)–(a). *Nontransforming viruses.*

III–A–2–a–(1)–(a)–(*1*). *Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (Strain FL and Plant Viruses. (40)* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(1)–(a)–(*2*). *Hepatitis B.*

III–A–2–a–(1)–(a)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for purified subgenomic DNA segments. (*38*)

III–A–2–a–(1)–(a)–(*2*)–(*b*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(a)–(*2*)–(*c*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(a)–(*3*). *Other Nontransforming Member of Presently Classified Viral Families.(36)*

III–A–2–a–(1)–(a)–(*3*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA(*38*) segments or (ii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(a)–(*3*)–(*b*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(b). *Transforming Viruses.(37A)*

III–A–2–a–(1)–(b)–(*1*). *Herpes Saimiri, Herpes Ateles, and Epstein Barr Virus.(39)*

III–A–2–a–(1)–(b)–(*1*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(1)–(b)–(*1*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene(*38*) or (ii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(b)–(*1*)–(*c*). P3 physical containment + an HV1 host-vector, or P2 + HV2, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(b)–(*2*). *Other Transforming Members of Presently Classified Viral Families.(36)*

III–A–2–a–(1)–(b)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments(*38*)

III–A–2–a–(1)–(b)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) subgenomic DNA segments containing an entire transforming gene, (iii) purified cDNA copies of viral mRNA,(*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2). *DNA Transcripts of RNA Viruses.*

III–A–2–a–(2)–(a). *Retroviruses.*

III–A–2–a–(2)–(a)–(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses.(39)*

III–A–2–a–(2)–(a)–(*1*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(2)–(a)–(*1*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic DNA segments(*38*) containing an entire transforming gene.

III–A–2–a–(2)–(a)–(*1*)–(*c*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) purified cDNA copies of viral mRNA,(*37*) or (iii) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(a)–(*2*). *Other Members of the Family Retroviridiae.(36)*

III–A–2–a–(2)–(a)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(2)–(a)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) subgenomic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA, (*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(b). *Negative Strand RNA Viruses.* Pl physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(2)–(c). *Plus-Strand RNA Viruses.*

III–A–2–a–(2)–(c)–(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(2)–(c)–(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families. (36)*

III–A–2–a–(2)–(c)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments. (*38*)

Table III.—Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D

[See text for full details]

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic (38)		Genomic¹		cDNA from viral mRNA (37)
	Non-transforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
DNA:					
Nontransforming viruses:					
AAV, MVM, mouse adeno (strain FL)	P1 + HV1	....	P1 + HV1	....	P1 + HV1
Plant viruses	P1 + HV1	....	P1 + HV1	....	P1 + HV1
Hepatitis B	P1 + HV1(38)	....	P2 + HV2 or P3 + HV1	....	P2 + HV1
Other	P1 + HV1(38)	....	P1 + HV1	....	P1 + HV1
Transforming viruses:					
Herpes Saimiri, H. Ateles and EBV (39)	P1 + HV1(38)	P2 + HV1	P2 + HV2 or P3 + HV1	....	P2 + HV1

Table III.—*Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D—Continued*

[See text for full details]

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic [38]		Genomic [1]		cDNA from viral mRNA [37]
	Non-transforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
Other	P1 + HV1[38]	P2 + HV1	P2 + HV1	—	P2 + HV1
RNA:					
Retroviruses:					
Gibbon ape, wooly monkey FeLV and FeSV [39].	P1 + HV1[38]	P2 + HV1	P2 + HV2 or P3 + HV1	—	P2 + HV2 or P3 + HV1
Other	P1 + HV1[38]	P2 + HV1	P2 + HV1	—	P2 + HV1
Negative-Strand RNA	P1 + HV1	—	P1 + HV1	P1 + HV1	P1 + HV1
Plus-Strand RNA:					
Types 1 and 2 Sabin polio, 17D yellow fever vaccine strains.	P1 + HV1	—	P1 + HV1	—	P1 + HV1
Other	P1 + HV1[38]	—	P2 + HV1	—	P2 + HV1
Double-stranded RNA	P1 + HV1	—	—	P1 + HV1	P1 + HV1
Plant viruses + viroids	P1 + HV1	—	P1 + HV1	P1 + HV1	P1 + HV1
Intracellular viral DNA	[2]	[2]	[2]	—	

[1] See exception given at asterisk at end of appendix D.

[2] See text.

III–A–2–a–(2)–(c)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, or (ii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(2)–(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(2)–(e). *RNA Plant Viruses and Plant Viroids.* (*48*) P1l physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see Section III–A–(1)–(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III–A–2–b. *Eukaryotic Organelle DNAs.* P2 phycial containment + an HV1 host-vector, or P1l + HV2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III–A–2–c. *Prokaryotic Plasmid and Phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages (See Section III–A–1–b.)

III–A–3. *Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone made from a random assortment of DNAs has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified (*41*) and in which the absence of harmful sequences has been established (*3*) can be carried out under lower containment conditions than used for the corresponding shotgun experiment.(*42*). The containment may be decreased one step in physical containment (P4 + P3; P3 + P2; P2 + P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3 + HV2; HV2 + HV1) while maintaining the specified physical containment. The Institutional Biosafety Committee (IBC) must review such a reduction and the approval of the IBC and of the NIH must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1 + HV1, which requires prior NIH approval. (See Section IV–E–1–b–(3)–(e).)

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (*3*) experiments involving this recombinant DNA may be carried out under lower containment conditions. Institutional Biosafety Committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1 + HV1, or reduction of containment levels by more than one step, which also requires prior NIH approval. (See Section IV–E–1–b–3–(e).)

III–B. *Experiments with Prokaryotic Host-Vectors Other Than E. coli K-12*

III–B–1. *HV1 and HV2 Systems.* Certain certified HV1 and HV2 host-vector systems appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, the classification of containment levels for experiments using them will be assigned by NIH.

III–B–2. *Return of DNA Segments to Prokaryotic Non-HV1 Host of Origin.* Certain experiments involving those prokaryotes that echange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4). For a prokaryote which can exchange genetic information(*35*) with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host B and progagated in Host B under P1 conditions.(*43*)

III-B-3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(c), and IV-E-1-b-(3)-(b).)

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment.(*2A*) Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations (See Section IV-E-1-b-2-(f)).

III-C. *Experiments with Eukaryotic Host-Vectors.*

III-C-1. *Vertebrate Host-Vector System.* (*44*) The subsections of Sections III-C-1-a, -b, -c and -d involve the use of specific viral vectors, namely polyoma, SV40, human adenoviruese 2 and 5, and mouse adenovirus strain FL, respectively. The subsections of Section III-C-1-e involve the use of all viral vectors including the specific viral vectors considered in the subsections of Sections III-C-1-a, -b, -c and -d, as well as any other viral vector. When the reader finds that the containment level given for specific experiment in a subsection of Section III-C-1-e is different from the containment level given in a subsection of Section III-C-1-a, -b, -c or -d, he may choose which of the two containment levels he wishes to use for the experiment.

III-C-1-a. *Polyoma Virus.*

III-C-1-a-(1). *Productive Virus-Cell Interactions.*

III-C-1-a-(1)-(a). Defective or whole polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III-C-1-a-(1)-(a)-(*1*). from bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins; (*34*)

III-C-1-a-(1)-(a)-(*2*). from mice;

III-C-1-a-(1)-(a)-(*3*). from eukaryotic organisms that do not produce potent polypeptide toxins, (*34*) provided that the DNA segment is > 99% pure.

III-C-1-a-(1)-(b). Defective polyoma genomes with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.(*34*)

III-C-1-a-(1)-(C). Whole virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.(*34*).

III-C-1-a-(1)-(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-a-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole polyoma virus genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the precribed physical and biological containment conditions (See Section IV-E-1-b-(3)-(c).)

III-C-1-b. *Simian Virus 40.*

III-C-1-b-(1). *Productive Virus-Cell Interactions.*

III-C-1-b-(1)-(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-b-(1)-(a)-(*1*). bacteria of Class 1 or Class 2,(*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins;(*34*)

III-C-1-b-(1)-(a)-(*2*). unifected African green monkey kidney cell cultures.

III-C-1-b-(1)-(b). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins(*34*) (Shotgun experiments or purified DNA).

III-C-1-b-(1)-(c). Experiments involving the use of defective SV40 genomes to progagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-b-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole SV40 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNS sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c. *Human Adenoviruses 2 and 5.*

III-C-1-c-(1). *Productive Virus-Cell Interactions.*

III-C-1-c-(1)-(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two essential genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III-C-1-c-(1)-(a)-(*1*). Bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent ploypeptide toxins; (*34*).

III-C-1-c-(1)-(a)-(2). Eukaryotic organisms that do not produce potent polypeptide toxins (*34*) (shotgun experiments or purified DNA).

III-C-1-c-(1)-(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d. *Murine Adenovirus Strain FL.*

III-C-1-d-(1). *Productive Virus-Cell Interactions.*

III-C-1-d-(1)-(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-d-(1)-(a)-(*1*). Bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent polypeptide toxins; (*34*).

III–C–1–d–(1)–(a)–(*2*). Eukaryotic organisms that do not produce potent polypeptide toxins (*34*) (shotgun experiments or purified DNA).

III–C–1–d–(1)–(b). Experiments involving the use of whole murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(1)–(c). Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole murine adenovirus strain FL genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–e. All Viral Vectors.

III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:

III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more that two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (*36*) being considered identical (*50*)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with a helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents *(1)*.

III–C–1–e–(2). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

NIH will also review on a case-by-case basis *(45)* all experiments involving the use of virus vectors in animals and will prescribe the physical and biological containment conditions appropriate for such studies. (See Section IV–E–1–b–(3)–(c).)

III–C–1–f. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that hose (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experiments which require the use of the whole animals will be evaluated by NIH on a case-by-case basis. *(45)*

III–C–2. *Invertebrate Host-Vector Systems.*

III–C–2–a. *Insect Viral Vectors.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, and integration of the viral DNA in vertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the recommended physical containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–2–b. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (See Section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P1 physical containment for invertebrate cells in culture since invertebrate cells in culture inherently exhibit a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis. *(45)*

III–C–3. *Plant Viral Host-Vector Systems. (48)* The DNA plant viruses which could currently serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosiac Virus (CaMV) and its close relatives *(2A)* which have relaxed circular double-stranded DNA genomes with a molecular weight of 4.5 × 10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small ($<10^6$ daltons) single-stranded DNA genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants shall be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-restrictive, preferably with positive air pressure, *(2A)* and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected materials shall be removed from the greenhouse or cabinet in sealed insect-proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary.

Infected plant materials which have to be removed from the greenhouse or cabinet for further research shall be maintained under insect-restrictive conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The viruses or their DNA may also be useful as vectors to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses provides adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is necessary, except as described in the following paragraph.

Experiments involving the use of plant genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-4. *Plant Host-Vector Systems Other than Viruses. (48)* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these guidelines (see Section I-E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit *(2A)* a decrease in the physical containment to P1.

Intact plants or propagative plant parts which cannot be grown in a standard P2 laboratory because of their large size may be grown under the P1 conditions described above in Section III-C-3, except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work, and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules.

III-C-5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.*

Certain certified HV1 and HV2 host-vector system appear in Appendix D. The containment levels for these systems are given in the subsections of Section III-A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, they may be added to Appendix D (and thus the containment levels for their use will be those of the subsections of Section III-A). Alternatively, at the time of their certification, another classification of containment levels for experiments using them may be assigned by NIH.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector and propagated in *E. coli* K-12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. *(43)*

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(c), and IV-E-1-b-(3)-(b).)

III-C-6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a vector and propagated in *E. coli* K-12 under P1 containment conditions. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. *(2A)*

III-C-7. *Transfer of cloned DNA Segments to Eukaryotic Organisms.*

III-C-7-a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source [Section I-D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* under P1 conditions, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study *(2A)*. Transfers to any other host will be considered by the RAC on a case-by-case basis *(45)*.

III-C-7-b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I-D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under P1 conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and progagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (*2A*). Intact plants or propagative plant parts may be grown undr P1 conditions described undr Section III-C-3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis (*45*).

III-D. *Complementary DNAs.* Specific containment levels are given in Section III-A-2-a (see also last column of Table III) for complementary DNA (cDNA) of viral mRNA. For the other Sections of the Guidelines, where applicable, cDNAs synthesized *in vitro* are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under III-A-1, shotgun experiments; cDN's formed from purified and characterized RNAs are included under III-A-3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III-E. *Synthetic DNAs.* If the synthetic DNA segment is likely to (*2A*) yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product,(2A) it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment which corresponds to a nonharmful gene of birds, to be propagated in *Saccharomyces cerevisiae,* would require P2 physical containment plus an HVI host-vector, or P1 + HV2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecule are exempt (*4*) from the Guidelines.

IV. Roles and Responsibilities

IV-A. *Policy.* Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgement are the key essentials to protection of health and the environment.

The Guidelines are intended to help the Institution, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the *purpose* of the Guidelines as well as to their speifics.

Each Institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV–B. *General Applicability.* The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research performed by NIH directly.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an Institution that can and does assume the responsibilities assigned in these Guidelines.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

IV–C. *General Definitions.* The following terms, which are used throughout the Guidelines, are defined as follows:

IV–C–1. "DNA" means deoxyribonucleic acid.

IV–C–2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules which result from the replication of a molecule described in (i) above.

IV–C–3. (Deleted)

IV–C–4. "Institution" means any public or private entity (including Federal, State, and local government agencies).

IV–C–5. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV–D–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV–D–2 and –3.

IV–C–6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV–E–3.

IV–C–7. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specific in Section IV–E–2.

IV–C–8. "Director, NIH" or "Director" means the Director of the National Institutes of Health and any other officer or employee of NIH to whom authority has been delegated.

IV–C–9. "Federal Interagency Advisory Committee on Recombinant DNA Research" means the committee established in October 1976 to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH, on the coordination of those aspects of all Federal programs and activities which relate to recombinant DNA research.

IV–C–10. "Administrative Practices Supplement" or "APS" means a publication to accompany the NIH Guidelines specifying administrative procedures for use at NIH and at Institutions.

IV–C–11. "Laboratory Safety Monograph" or "LSM" means a publication to accompany the NIH Guidelines describing practices, equipment, and facilities in detail.

IV–D. *Responsibilities of the Institution.*

IV–D–1. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV–D–1–a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution, as part of its general responsibilities for implementing the Guidelines, may establish additional procedures, as deemed necessary, to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include (i) statements formulated by the Institution for general implementation of the Guidelines and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV–D–1–b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV–D–2 and carries out the functions detailed in Section IV–D–3.

IV–D–1–c. (Deleted)

IV–D–1–d. (Deleted)

IV–D–1–e. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section> IV–D–4.

IV–D–1–f. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV–D–5, and assist investigators to do so.

IV–D–1–g. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC.

IV–D–1–h. Determine the necessity, in connection with each project, for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The Laboratory Safety Monograph (LSM) discusses various possible components of such a program—for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness.]

IV–D–1–i. Report within 30days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV–D–2. *Membership and Procedures of the IBC.* The Institution shall establish an Institutional Biosafety Committee (IBC) meeting the following requirements:

IV-D-2-a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any poitential risk to public health or the environment. At least two members (but not less than 20 percent of the membership of the committee) shall not be affiliated with the Institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community The Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member (see Section IV-D-4).

IV-D-2-b. In order to ensure the professional competence necessary to review recombinant DNA activities, it is recommended that (i) the IBC include persons from disciplines relevant to recombinant DNA technology, biological safety, and engineering; (ii) the IBC include, or have available as consultants, persons knowledgeable in institution commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be a nondoctoral person from a laboratory technical staff.

IV-D-2-c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require. (See the Administrative Practice Supplement for further guidance.)

IV-D-2-d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

IV-D-2-e. The Institution may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section IV-D-3-a.)

IV-D-2-f. Central to implementation of the Guidelines is the review of experiments by the IBC. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement.

IV-D-2-g. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection of privacy and proprietary interests.

IV-D-2-h. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public (e.g., reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV-D-3. *Functions of the IBC.* On behalf of the Institution, the IBC is responsible for:

IV-D-3-a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. This review shall include:

IV-D-3-a-(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV-D-3-a-(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

Note.—See Laboratory Safety Monograph (pages 187-190) for suggested guidance in conducting this review.

IV-D-3-b. Notifying the Principal Investigator (PI) of the results of their review.

IV-D-3-c. Reviewing periodically recombinant DNA research being conducted at the Institution, to ensure that the requirements of the Guidelines are being fulfilled.

IV-D-3-d. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

Note.—Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph. Included are information and references on decontamination and emergency plans. NIH and the Centers for Disease Control are available to provide consultation, and direct assistance if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant reseach-related illness or accident that appears to be a hazard to the public health.

IV-D-3-e. Reporting within 30 days to the appropriate institutional official and to the NIH Office of Recombinant DNA Activities (ORDA) any significant problems with or violations of the Gidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV-D-3-f. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH, (with the advice of the RAC when required) establishes the containment requirement.

IV-D-3-g. Performing such other functions as may be delegated to the IBC under Section IV-D-1.

IV-D-4. *Biological Safety Officer.* The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV-D-4-a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV-D-4-b. Reporting to the IBC and the Institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware, unless the BSO determines that the Principal Investigator (PI) has done so;

IV-D-4-c. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV-D-4-d. Providing advice on laboratory security;

IV-D-4-e. Providing technical advice to the PI and the IBC on research safety procedures.

Note.—See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV-D-5. *Principal Investigator.* On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV-D-5-a. *PI—General.* As part of this general responsibility, the PI shall:

IV-D-5-a-(1). Initiate or modify no recombinant DNA research subject to the Guidelines until that research, or the proposed modification thereof, has been approved by the Institutional Biosafety Committee (IBC) and has met all other requirements of the Guidelines and the Administrative Practices Supplement (APS).

(Note.—No prior approval by the IBC is required for most experiments described in Section III–O. Modify containment and experimental protocol according to recommendations of the IBC.)

IV–D–5–a–(2). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV–D–5–a–(3). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV–D–5–a–(4). Be adequately trained in good microbiological techniques;

IV–D–5–a–(5). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV–D–5–a–(6). Comply with shipping requirements for recombinant DNA molecules. (See Section II–C for shipping requirements, Laboratory Safety Monograph for technical recommendations, and the APS for administrative instructions and procedures. The requesting laboratory must be in compliance with the NIH Guidelines and under appropriate review by its IBC, and the sending investigator must maintain a record of all shipments of recombinant DNA materials.)

IV–D–5–b. *Submissions by the PI to NIH.* The PI shall:

IV–D–5–b–(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV–D–5–b–(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines (see Sections I–E–4 and I–E–5 and, for additional information on procedures, the APS); and

IV–D–5–b–(3). Petition NIH, with concurrence of the IBC, for exceptions to the prohibitions under these Guidelines (see Section I–D and, for additional information on procedures, the APS).

IV–D–5–b–(4). Petition NIH for determination of containment for experiments requiring case-by-case review.

IV–D–5–b–(5). Petition NIH for determination of containment for experiments not covered by the Guidelines.

IV–D–5–c. *Submissions by the PI to the IBC.* The PI shall:

IV–D–5–c–(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV–D–5–c–(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV–D–5–c–(3). Submit the initial research protocol (and also subsequent changes—e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval, and

IV–D–5–c–(4). Remain in communication with the IBC throughout the conduct of the project.

IV–D–5–d. *PI Responsibilities After Approval but Prior to Initiating the Research.* The PI is responsible for:

IV–D–5–d–(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV–D–5–d–(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV–D–5–d–(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV–D–5–e. *PI Responsibilities During the Conduct of the Approved Research.* The PI is responsible for:

IV–D–5–e–(1). Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

IV–D–5–e–(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV–D–5–e–(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV–D–5–e–(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, and genotypic and phenotypic characteristics); and

IV–D–5–e–(5). *Publications.* PIs are urged to include, in all publications reporting on recombinant DNA research, a description of the physical and biological containment procedures employed.

IV–E. *Responsibilities of NIH.*

IV–E–1. *Director.* The Director, NIH, is responsible for (i) establishing the NIH Guidelines on recombinant DNA research, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has a number of responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV–E–1–a. *General Responsibilities of the Director, NIH.* The responsibilities of the Director shall include the following:

IV–E–1–a–(1). Promulgating requirements as necessary to implement the Guidelines;

IV–E–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–E–2. The RAC's membership is specified in its charter and in Section IV–E–2;

IV–E–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–E–3; and

IV–E–1–a–(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW, for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC.

IV–E–1–b. *Specific Responsibilities of the Director, NIH.* In carrying out the responsibilities set forth in this Section, the Director shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV–E–1–b–(1). *The Director is responsible for the following major actions* (For these, the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the **Federal Register** at least 30 days before the meeting, and the Director will also publish the proposed actions in the **Federal Register** for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days of comment before final action is taken. The Director's final decision, along with response to the comments, will be published in the **Federal Register** and the *Recombinant DNA Technical Bulletin.* The RAC and IBC chairpersons will be notified of this decision):

IV–E–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(c). Certifying new host-vector systems, with the exception of minor modifications of already certified systems. [The standards and procedures for certification are described in Section II–D–2–a. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment. See the Administrative Practices Supplement (APS) for further information]:

IV–E–1–b–(1)–(d). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment (see Sections I–E–4 and –5 and the APS for further information);

IV–E–1–b–(1)–(e). Permitting exceptions to the prohibited experiments in the Guidelines, in order, for example, to allow risk-assessment studies; and

IV–E–1–b–(1)–(f). Adopting other changes in the Guidelines.

IV–E–1–b–(2). *The Director is also responsible for the following lesser* actions (For these, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the Recombinant DNA Technical Bulletin):

IV–E–1–b–(2)–(a). Interpreting and determining containment levels, upon request by ORDA;

IV–E–1–b–(2)–(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV–E–1–b–(2)–(c). Assigning containment levels for experiments not explicitly considered in the Guidelines (see Section III);

IV–E–1–b–(2)–(d). Designating certain class 2 agents as class 1 for the purpose of these Guidelines (see Footnote 1 and Appendix B);

IV–E–1–b–(2)–(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms(*1*) and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens beyond that which occurs by natural genetic exchange; and

IV–E–1–b–(2)–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (see Section III–B–3).

IV–E–1–b–(3). *The Director is also responsible for the following actions.* (The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin*):

IV–E–1–b–(3)–(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV–E–1–b–(3)–(b). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–E–1–b–(2)–(c).

IV–E–1–b–(3)–(c). Determining appropriate containment conditions upon case-by-case analysis of experiments explicity considered in the Guidelines but for which no containment levels have been set (see Footnote 45 in Part V; Sections III–C–1–a through –e; and Sections III–C–2 and –3);

IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinat DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6);

IV–E–1–b–(3)–(e). Lowering containment levels for characterized clones or purified DNA (see Sections III–A–3–a and –b, and Footnotes 3 and 41);

IV–E–1–b–(3)–(f). Approving minor modifications of already certified host-vector systems. (The standards and procedures for such modifications are described in Section II–D–2); and

IV–E–1–b–(3)–(g). Decertifying already certified host-vector systems.

IV–E–1–b–(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV–E–1–b–(5). The Director, at the end of 36 months from the time these Guidelines are promulgated, will report on the Guidelines, their administration, and the potential risks and benefits of this research. In doing so, the Director will consult with the RAC and the Federal Interagency Committee. Public comment will be solicited on the draft report and taken into account in transmitting the final report to the Assistant Secretary for Health and the Secretary, HEW.

IV–E–2. *Recombinant Advisory Committee.* The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities, Bethesda, Md. 20205.

All meetings of the RAC will be announced in the **Federal Register**, including tentative agenda items, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV–E–1–b–(1) may be added to an agenda after it appears in the **Federal Register.**

IV–E–2–a. *The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–E–1–b–(1)* and *–(2).*

IV–E–3. *The Office of Recombinant DNA Activities.* ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Biological Safety Committee, Principal Investigators, Federal agencies, State and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV–E–1–b–(3). In addition, ORDA shall be responsible for the following:

IV–E–3–a. Review and approval of Institutional Biosafety Committee (IBC) membership;

IV–E–3–b through IV–E–3–c–(3). (Deleted)

IV–E–3–c–(4). Publish in the **Federal Register**:

IV–E–3–c–(4)–(a). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agenda 30 days in advance, with publication of the Director's proposed decision for 30 days of public and Federal agency comment followed by a published response, on any action listed in Section IV–E–1–(b)–(1); and

IV–E–3–c–(4)–(b). Announcements of RAC meetings and agendas 30 days in advance on any action listed in Section IV–E–1–b–(2).

Note.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV–E–3–c–(5). Publish the *Recombinant DNA Technical Bulletin;* and

IV–E–3–c–(6). Serve as executive secretary to the RAC.

IV–E–4. *Other NIH Components.* Other NIH components shall be responsible for:

IV–E–4–a. (Deleted)

IV–E–4–b. Certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary; and

IV–E–4–c. Announcing and distributing certified HV2 and HV3 host-vector systems (see Section II–E–3).

(See Administrative Practices Suplement for additional information on the administrative procedures of ORDA and other NIH components.)

IV–F. (Deleted)

IV–G. *Compliance.* As a condition for NIH funding of recombinant DNA research, institutions must ensure that such research conducted at or sponsored by the Institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV–G–1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–3. Information concerning noncompliance with the Guidelines may be brought foward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution, generally through the IBC, shall take appropriate action. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV–G–4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHEW and Public Health Service procedures shall govern.

IV–G–5. *Voluntary Compliance.* Any individual, corporation, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines, through the procedures set forth in Part VI.

V. Footnotes And References

1. The reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. The list of organisms in each class, as given in this publication, is reprinted in Appendix B to these Guidelines.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as Class 2 in the *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974, as Class 1 agents for the Purposes of these Guidelines (See section IV–E–1–b–(2)–(d)). An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

2A. In Parts I and III of the Guidelines, there are a number of places where Judgments are to be made. These include: "cells known to be infected with such agents" (Section I–D–1) "toxins potent for vertebrates" (Section I–D–2); "known to acquire it naturally" (Section I–D–5); "known to produce a potent polypeptide toxin * * * or known to carry such pathogens * * * not likely to be a product of closely linked eukaryote genes * * * shown not to contain such agents" (Section III–A–1–a–(5)–(a)); "shown to be free of disease causing microorganisms" (Section III–A–1–a–(5)–(b)); "close relatives" (Section III–C–3); and "procduce a potent polypeptide toxin" (Footnote 34).

In all these cases the principal investigator is to make the initial judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV–D–7–a). In all these cases, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV–D–3–a–(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV–E–3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interpreting and determining containment levels upon request by ORDA" (Section IV–E–1–b–(2)–(a)).

3. The following types of data should be considered in determining whether DNA recombinants are "characterize" and the absence of harmful sequences has been established: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or the host or be detrimental to humans, animals, or plants); (b) the type(s) of genetic information on the cloned sedment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuckease fragmentation analysis where applicable); (d) the genetic stabillity of the cloned fragment; and (e) any alterations in the biological properties of the vector and host.

4. In Section I–E, "exemptions" from the Guidelines are discussed. Such experiments are not covered by the Guidelines and need not be registered with NIH. In Section I–D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that any experiment may be expressly released from a prohibition. At that time it will be assigned an appropriate level of physical and biological containment.

5. Care should be taken to inactivate recombinant DNA before disposal. Procedures for inactivating DNA can be found in the "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research."

6. *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health, Education, and Welfare Publication No. CDC 75–8118.

1. *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

8. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

9. *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health.

10. *Biohazards in Biological Research* (1973). A. Hellman, M. N Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

11. *Handbook of Laboratory Safety* (1971). Second Edition. N. V. Steere (ed.). The Chemical Rubber Co., Cleveland.

12. Bodily, J. L. (1970). *General Administration of the Laboratory.* H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.). Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

13. Darlow, H. M. (1969). *Safety in the Microbiological Laboratory.* In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc. New York. pp. 169–204.

14. *The Prevention of Laboratory Acquired Infection* (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

15. Chatigny, M. A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures.* In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

16. *Design Criteria for Viral Oncology Research Facilities* (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75–891.

17. Kuehne, R. W. (1973). *Biological Containment Facility for Studying Infectious Disease.* Appl. Microbiol. 26–239–243.

18. Runkle, R. S., and G. B. Phillips (1969). *Microbial Containment Control Facilities.* Van Nostrand Reinhold, New York.

19. Chatigny, M. A., and D. I. Clinger (1969). *Contamination Control in Aerobiology.* In R. L. Dimmick and A. B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194–263.

19A. Horsfall, F. L., Jr., and J. H. Baner (1940). *Individual Isolation of Infected Animals in a Single Room* J. Bact. *40,* 569–580.

20. Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *Class I* is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

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29. Murray, N. E., and K. Murray (1974). *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments.* Nature *251,* 476–481.

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32. Donoghue, D. J., and P. A. Sharp (1977). *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance,* Gene *1,* 209–227.

33. Leder, P., D. Tiemeier and L. Enquist (1977). *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The gt WES System.* Science *196.* 175–177.

33A. Skalka, A. (1978). *Current Status of Coliphage EK2 Vectors.* Gene *3,* 29–35.

33B. Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein (1978). *Standardized Laboratory Tests for EK2 Certification.* Gene *3,* 36–38.

34. We are specifically concerned with the remote possibility that potent toxins could be produced by acquiring a single gene or cluster of genes. See also footnote 2A.

35. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I-E-4.

36. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews, Ed. Intervirology 12 (129–296) 1979. (As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

37. The cDNA copy of the viral mRNA must be >99% pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

37A. For the purpose of these Guidelines, viruses of the families *Papovavirida, Adenoviridae,* and *Herpetoviridae* (36) should be considered as "transforming" viruses. While only certain of these viruses have been associated with cell transformation *in vivo* or *in vitro,* it seems prudent to consider all members to be potentially capable of transformation. In addition, those viruses of the family *Poxviridae* that produce proliferative responses—i.e., myxoma, rabbit and squirrel fibroma, and Yaba viruses-should be considered as "transforming."

38. ≥99% pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.

39. The viruses have been classified by NCI as "moderate-risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recommendations on handling the viruses themselves.

40. (Deleted)

41. The DNA preparation is defined as "purified" if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

42. The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10^5-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

43. This is not permitted, of course, if it falls under any of the Prohibitions of Section I-D. Of particular concern here is prohibition I-D-5, i.e., "Deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture."

44. Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. It should be pointed out that risk of laboratory-acquired infection as a consequence of tissue culture manipulation is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, there should be a clear idea of the location within the molecule of:

(i) the sites at which DNA synthesis originates and terminates,

(ii) the sites that are cleaved by restriction endonucleases, and

(iii) the template regions for the major gene product.

If possible the helper virus genome should:

(i) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

45. Review of NIH on a case-by-case basis means that NIH must review and set appropriate containment conditions before the work may be undertaken. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin.*

46. Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

47. >90% pure; otherwise as for shotgun experiments.

48. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

49. A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

50. i.e., the total of all genomes within a Family shall not exceed two-thirds of the genome.

VI. Voluntary Compliance

VI–A. *Basic Policy.* Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I–IV of the Guidelines. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I–IV are provided below, in order to address these concerns.

VI–B. *IBC Approval.* The NIH Office of Recombinant DNA Activities (ORDA) will review the membership of an institution's Institutional Biosafety Committee (IBC) and, where it finds the IBC meets the requirements set forth in Section IV–D–2, will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member for purposes of Section IV–D–2–a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has a direct financial interest, so long as the member has not been and does not expect to be engaged in the project. Section IV–D–2–d is modified to that extent for purposes of these institutions.

VI–C. (Deleted)

VI–D. *Certification of Host-Vector Systems.* A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Section II–D–2–a.

Institutions not otherwise covered by the Guidelines will not be subject to Section II–D–3 by complying with these procedures.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–E. *Requests for Exceptions, Exemptions, Approvals.* Requests for exceptions from prohibitions, exemptions, or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I–IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exception, exemption, or other approval which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–F. *Protection of Proprietary Data.* In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, [or processes * * * of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI–F–1. In submitting information to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets or privileged or confidential commercial or financial information.

VI–F–2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

VI–F–3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised, except to the extent that earlier release, in the judgement of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI-F-4. Projects should be registered in accordance with procedures specified in the *Administrative Practices Supplement*. The following information will usually be considered publicly available information, consistent with the need to protect proprietary data:

a. The names of the institution and principal investigator.

b. The location where the experiments will be performed.

c. The host-vector system.

d. The source of the DNA.

e. The level of physical containment.

VI-F-5-a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI-F-5-b. A request for presubmission review should be submitted to ORDA, along with the records involved. These records must be clearly marked as being the property of the institution, on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. ORDA will then seek a determination from the HEW Freedom of Information Officer, the responsible official under HEW regulations (45 CFR Part 5), as to whether the records involved (or some portion) are or are not available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI-F-5-c. ORDA will inform the institution of the HEW Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HEW, NIH, or ORDA.

VI-F-5-d. The HEW Freedom of Information Officer's determination will represent that official's judgement, as of the time of the determination, as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act, if at the time of the determination the records were in ORDA files and a request was received from them under the Act.

Appendix A—Exemptions Under I-E-4

Section I-E-4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I-E-4 of these revised Guidelines are recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagated in any of the organisms within a sublist. (Classification of *Bergey's Manual of Determinative Bacteriology*, eighth edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

Sublist A

1. Genus *Escherichia*
2. Genus *Shigella*
3. Genus *Salmonella* (including *Arizona*)
4. Genus *Enterobacter*
5. Genus *Citrobacter* (including *Levinea*)
6. Genus *Klebsiella*
7. Genus *Erwinia*
8. *Pseudomonas aeruginosa, Pseudomonas putida* and *Pseudomonas fluorescens*
9. *Serratia marcescens*

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*
6. *Bacillus nato*
7. *Bacillus amyloliquefaciens*
8. *Bacillus aterrimus*

Sublist C

1. *Streptomyces aureofaciens*
2. *Streptomyces rimosus*
3. *Streptomyces coelicolor*

Sublist D

1. *Streptomyces griseus*
2. *Streptomyces cyaneus*
3. *Streptomyces venezuelae*

Sublist E

One way transfer of *Streptococcus mutans* DNA into *Streptococcus sanguis*.

Sublist F

1. *Streptococcus sanguis*
2. *Streptococcus pneumoniae*

Appendix B—Classification of Micro-Organisms on the Basis of Hazard

I. Classification of Etiologic Agents on the Basis of Hazard (*1*)

A. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

B. Class 2 Agents

1. Bacterial Agents

Actinobacillus—all species except A. *mallei*, which is in Class 3

Arizona hinshawii—all serotypes

Bacillus anthracis

Bordetella—all species

Borrelia recurrentis, B. vincenti

Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani

Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis C. pyogenes, C. renale

Diplococcus (Streptococcus) pneumoniae

Erysipelothrix insidiosa

Escherichia coli—all enteropathogenic serotypes

Haemophilus ducreyi, H. influenzae

Herellae vaginicola

Klebsiella—all species and all serotypes

Leptospira interrogans—all serotypes

Listeria—all species

Mima polymorpha

Moraxella—all species

Mycobacteria—all species except those listed in Class 3

Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae*, which are in Class 5

Neisseria gonorrhoeae, N. meningitidis

Pasteurella—all species except those listed in Class 3.

Salmonella—all species and all serotypes

Shigella—all species and all serotypes

Sphaerophorus necrophorus

Staphylococcus aureus

Streptobacillus moniliformis

Streptococcus pyogenes

Treponema carateum, T. pallidum, and T. pertenue

Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

2. Fungal Agents

***Actinomycetes* (including *Nocardia* species and *Actinomyces species*

and Arachnia propionica)
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

3. Parasitic Agents
Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

4. Viral, Rickettsial, and Chlamydial Agents
Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Corona viruses
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox, Monkey pox, and Whitepox,* which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus,* which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus,* which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. Class 3 Agents

1. Bacterial Agents
Actinobacillus mallei *
Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains *)
Pseudomonas pseudomallei *
Yersenia pestis

2. Fungal Agents
Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. Parasitic Agents
Schistosoma mansoni

4. Viral, Rickettsial, and Chlamydial Agents
****Alastrim, Smallpox, Monkey pox, and Whitepox,* when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Vesicular stomatitis virus *
Yellow fever virus—wild, when used *in vitro*

D. Class 4 Agents

1. Bacterial Agents: None
2. Fungal Agents: None
3. Parasitic Agents: None
4. Viral, Rickettsial, and Chlamydial Agents
****Alastrim, Smallpox, Monkey pox, and Whitepox,* when used for transmission or animal inoculation experiments
Hemorrhagic fever agents, including *Crimean hemorrhagic fever, (Congo), Junin,* and *Machupo* viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses*
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used for transmission or animal inoculation experiments

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (*2*)

A. Low-Risk Oncogenic Viruses
Rous Sarcoma
SV-40
CELO
Ad7-SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Avian Leukosis
Murine Leukemia
Murine Sarcoma
Mouse mammary tumor
Rat Leukemia
Hamster Leukemia
Bovine Leukemia
Dog Sarcoma
Mason-Pfizer Monkey Virus
Marek's
Guinea Pig Herpes
Lucke (Frog)
Adenovirus
Shope Fibroma
Shope Papilloma

B. Moderate-Risk Oncogenic Viruses
Ad2-SV40
FeLV
HV Saimiri
EBV
SSV-1
GaLV
HV ateles
Yaba
FeSV

III. Animal Pathogens (*3*)

A. Animal disease organisms which are forbidden entry into the United States by Law (CDC Class 5 agents)

1. Foot and mouth disease virus

B. Animal disease organisms and vectors which are forbidden entry into the United States by USDA Policy (CDC Class 5 Agents)
African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious

agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rhinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Footnotes and References of Appendix B

*A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD. 20782.

**Since the publication of the classification in 1974 (*1*), the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

***All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

1. *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.
2. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75–790.
3. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C—Exemptions Under I–E–5

Section I–E–5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines."

Under exemption I–E–5 of these Revised Guidelines are those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus).

Appendix D—HV1 and HV2 Host-Vector Systems Assigned Containment Levels as Specified in the Subsections of Section III–A

As noted above at the beginning of Section III–A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III–A. Those so classified as of publication of these Revised Guidelines are listed below.

* HV1—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:
(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.
(2) csp–1 strain UCLA37 and csp–2 strains FS 590, UCLA101 (these are conidial separation mutants).
(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—Asporogenic mutant derivatives of *B. subtilis.* These derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of HV1 systems based on these plasmids.

HV2—The asporogenic mutant derivative of *Bacillus subtilis*, ASB298, with the following plasmids as the vector component: pUB110, pC194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E—Actions Taken Under the Guidelines

As noted in the subsections of Sections IV–E–1–b–(1) and IV–E–1–b–(2), the Director, NIH, may take certain actions with regard to the Guidelines after consideration by the RAC.

Some of the actions taken to date include the following:

1. The following experiment has been approved: The cloning in *B. subtilis*, under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid vectors provided that an HV1 *B. subtilis* host is used.

2. Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HV1 *N. crassa* systems are approved provided that these are carried out at physical containment one level higher than required for HV1. However, if P3 containment is specified for HV1 *N. crassa*, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia, including the use of a biological safety cabinet.

3. P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for the species which are known to be pathogenic for man, animals, or plants.

4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HV1 systems (i.e. one step higher physical containment than that specified in the subsections of Section III–A). Transfer into plant parts or cells in culture would be permitted at the same containment level (one step higher).

5. *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae,* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

6. *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12 or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as vectors any plasmid indigenous to *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms.

7. Certain cloned segments of *Anabena* DNA may be transferred into *Klebsiella* under P2 physical containment.

8. Permission is granted to clone foot-and-mouth disease virus in the EK1CV host-vector system consisting of *E. coli* K–12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

9. Permission is granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under P1 + EK1 conditions

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* These follow the assigned containment levels as specified in the subsections of Section III–A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3 + HV1 or P2 + HV2 rather than the levels given in the subsections of Section III–A.

in *Escherichia coli* K-12 and under P1 conditions in *Pseudomonas aeruginosa.*

10. Permission is granted to return to the host of origin *Helminthosporanium maydis* (race O) DNA which has been cloned in yeast strain SHY2 using the hybrid *E. coli*—yeast plasmid Y1p5. The cloned DNA may be returned to, and propagated in, *Helminthosporanium maydis* at the P2 level of physical containment.

11. Permission is granted to return *Schizophyllum commune* DNA (or yeast DNA) cloned in *Saccharomyces cerevisiae* with YR or 2 mu circle vectors to *Schizophyllum commune.* The cloned DNA may be returned to, and propagated in, *Schizophyllum commune* at the P2 level of physical containment.

12. Permission is granted to return *Wangiella dermatitidis* DNA to *Wangiella dermatitidis* using an HV2 certified *Saccharomyces/E. coli* hybrid vector. The *Wangiella dermatitidis* may be propagated at the P3 level of physical containment.

13. Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Fransico, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.

14. *Saccharomycopsis lipolytica* may be used as a host for tranformation with defined *Escherichia coli/ Saccharomyces cerevisiae* hybrid plasmids and the hybrid plasmids may be used for cloning *S. lipolytica* DNA in *E. coli* and returning the cloned DNA to *S. lipolytica.*

15. Conjugative plasmids or transducing phages may be employed in recombinant DNA experiments when employing *E. coli* as host when a small defined segment of Adenovirus 2 DNA is employed as linker DNA.

16. Permission is granted to introduce DNA segments from aphid transmissible strains into non-aphid transmissible strains of Cauliflower mosaic virus in order to study the factors determining aphid transmissibility.

17. Permission is granted to return *Mucor racemosus* DNA which has been cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus.* In addition, permission is granted to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae sequences.* These manipulations may be performed under P2 conditions.

18. *Schizosaccharomyces pombe* DNA may be cloned in *Schizosaccharomyces pombe* using approved HV1 *Saccharomyces cerevisiae/E. coli* hybrid plasmids as vectors under P1 containment conditions.

19. The pyrogenic endotoxin type A (Tox A) gene of *Staphylococcus aureus* may be cloned in an HV2 *Bacillus subtilis* host-vector system under P3 containment conditions.

20. A hybrid plasmid composed of, (1) *E. coli* plasmid pBR325, (2) the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, (3) the thiamine gene of *E. coli,* and (4) *Arabidopsis* DNA, may be transformed into *Agrobacterium tumefaciens* under P1 conditions. The *Agrobacterium tumefaciens* may subsequently be used to introduce the composite plasmid carrying *Arabidopsis* DNA and the *E. coli* thiamine gene into *Arabidopsis* plants under P1 containment conditions.

21. *Chlamydomonas reinhardi* can be used as a host for cloning defined DNA segments derived from *E. coli* and *Saccharomyces cerevisiae* using *E. coli/ S. cerevisiae* hybrid vectors under P2 physical containment.

22. *Candida albicans* can be used as a host for cloning *Candida albicans* DNA following propagation of the DNA in *E. coli* K-12 or in *Saccharomyces cerevisiae* employing an *E. coli-S. cerevisiae* hybrid plasmid vector or the yeast 2 micron plasmid.

23. The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K-12 employing the non-conjugative *Haemophilus* plasmid, pRSF0885, under P1 conditions.

24. *Zymomonas mobilis* may be used as a host under P2 conditions for transformation by recombinant DNA derived from *Pseudomonas* strains that are non-pathogenic for animals or plants, and that has been cloned in an *E. coli* K-12 host.

25. Protoplasts of *Streptosporangium brasiliense* may be transformed with a hybrid plasmid containing pBR322 plus a *Streptosporangium* plasmid into which have been incorporated specified DNA segments from *Streptomyces* species or an HV1 approved *Bacillus subtilis* cloning vector.

Appendix F—Certified HV2 Host-Vector Systems

While the Guidelines no longer specify the use of *E. coli* K-12 EK2 or *Saccharomyces cerevisiae* HV2 systems, investigators may wish to employ these systems in specific instances. The currently certified EK2 and HV2 systems are:

HV2—The following sterile strains of *Saccharomyces cerevisiae,* all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

EK2 Plasmid Systems. The *E. coli* K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 systems based on bacteriophage lambda:

Vector	*Host*
-gt*WES.-B'*	DP50*supF*
-gt*WES.-B'*	DP50*supF*
-gtZJ*vir.-B'*	*E. coli* K-12
-gtALO.-B	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Dated: November 14, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government program contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its annoucements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A-95 because they fit the description of "program not considered appropriate" in Section 8-(b)-(4) and (5) of that Circular.

[FR Doc. 80-36316 Filed 11-20-80; 8:45 am]

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federal register

Thursday
January 31, 1980

Part IX

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Proposed Actions Under Guidelines; Recombinant DNA Advisory Committee; Meeting

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Wilson Hall, Building 1, Bethesda, Maryland 20205, on March 6, 1980, from 9:00 a.m. to recess at approximately 6:00 p.m. and on March 7, 1980, from 8:30 a.m. to 5:00 p.m. This meeting will be open to the public on March 6 from 9:00 a.m. to recess at approximately 6:00 p.m., and on March 7, from 8:30 a.m. to 3:00 p.m., to discuss:

Amendment of Guidelines.
Containment practices appropriate for large-scale production.
Exceptions to prohibitions.
Exemptions for organisms that exchange genetic information.
Procedures for lowering characterized clones.
Review of large-scale experiments.
E. coli K–12 host-vector systems.
Host-vector systems other than *E. coli* K–12.
NIH risk-assessment plan.
Review of protocols for required containment levels.
Other matters requiring necessary action by the Committee.

Attendance by the public will be limited to space available.

In accordance with the provisions set forth in section 552b(c)(4), Title 5, U.S. Code and section 10(d) of Pub. L. 92–463, the meeting will be closed to the public on March 7 from 3:00 p.m. to 5:00 p.m. for the review, discussion and evaluation of proposal(s) from a commercial concern(s) for scale-up of recombinant DNA experiments. The proposal(s) and the discussions could reveal confidential trade secrets or commercial property such as patentable material.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 4A52, telephone 301–496–6051, will provide materials to be discussed at the meeting, rosters of committee members and substantive program information. A summary of the meeting will be available at a later date.

Dated: January 25, 1980.

Suzanne L. Fremeau,
Committee Management Officer, National Institutes of Health.

[FR Doc. 80–3040 Filed 1–30–80; 8:45 am]
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Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHEW.

ACTION: Notice of proposed actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposals for actions to be taken under the 1978 NIH Guidelines for Research Involving Recombinant DNA Molecules [**Federal Register** of December 22, 1978 (43 FR 60108)]. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its March 6–7, 1980, meeting, the Director of the National Institutes of Health will issue decision on these proposals in accord with the Guidelines.

DATE: Comments must be received by March 3, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules (43 FR 60108), as well as actions under these Guidelines.

1. *Amendment of Section IV–D–2–a.* As a comment on the Proposed Revised Guidelines for Research Involving Recombinant DNA Molecules published for comment in the **Federal Register** on November 30, 1979 (44 FR 69210), David Lester of Princeton, New Jersey, has proposed that Section IV–D–2–a, dealing with membership and procedures for the Institutional Biosafety Committee, be modified by adding as the penultimate sentence of IV–D–2–a the following:

"Such nonaffiliated members shall be appointed by the governing body of the community in which the institution is situated."

2. *Amendment of Section III–O of the Proposed Revised Guidelines.* Dr. Kent Wilcox of the Medical College of Wisconsin has suggested a clarification of the language of Section III–O, Classification of Experiments Using the *E. coli* K–12 Host-Vector Systems, of the proposed revised Guidelines published for comment in the **Federal Register** on November 30, 1979 (44 FR 69210). Section III–O is proposed to read, in part, as follows:

> * * * For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E coli* K–12 efficiently express any gene coding for a eukaryotic protein * * *.

Dr. Wilcox suggests that the term "eukaryotic protein" is not well defined. He questions whether this term refers specifically to proteins encoded in the DNA of eukaryotic organisms and if so, whether animal viruses and viroids are considered to be eukaryotic organisms. Dr. Wilcox suggests that the last sentence of the above citation be modified to read as follows (modified text in italics):

> * * * An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express *as a protein product the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism* * * *.

3. *Amendment of Section III–O of the Proposed Revised Guidelines.* Dr. Stuart Levy of Tufts University School of Medicine has recommended an amendment to Section III–O of the Proposed Revised Guidelines (44 FR 69210). Dr. Levy notes that the plasmid studied in his risk-assessment studies was a nonconjugative poorly

mobilizable plasmid. He suggests that a distinction be made between nonconjugative plasmids and nonconjugative plasmids which are also poorly mobilizable; such an amendment would provide additional biological containment. The relevant portion of Section III–O of the Proposed Revised Guidelines which would be amended reads as follows:

> * * * Other experiments using *E. coli* K–12 shall use P1 physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system (i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors) * * *.

The amended section proposed by Dr. Levy would read as follows (amended text in italic):

> * * * Other experiments using *E. coli* K–12 shall use P1 physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system (i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) *lambda or lambdoid bacteriophages or nonconjugative poorly mobilizable (able to be mobilized at a frequency of less than* 10^{8} *by a derepressed conjugative plasmid in the same cell) plasmids shall be used as vectors)* * * *

4. *Request to Eliminate Section I–D–3 from the Guidelines.* In response to the proposed revised Guidelines published in the **Federal Register** on November 30, 1979 (44 FR 69210), Dr. Clarence Kado of the University of California, Davis, has asked the NIH to consider deleting from the Guidelines Section I–D–3 which reads as follows:

> Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange.[2A]

Dr. Kado explains his arguments for elimination of the prohibition in a letter to ORDA.

5. *Request to Include all Species of the Genus Erwinia in Appendix A, Sublist A.* In response to the proposed revised Guidelines published in the **Federal Register** on November 30, 1979 (44 FR 69210), Dr. Clarence Kado of the University of California, Davis, has requested that the NIH consider the inclusion of all *Erwinia* species in Sublist A, Appendix A. Currently, only one *Erwinia* species, *E. amylovora*, is included on the list. Dr. Kado suggests that consideration be given to all *Erwinia* species on the basis of an exchange of genetic material by natural means with *E. coli* and a 20% DNA–DNA homology of *E. coli* with *Erwinia* species.

6. *Classification of Plant Pathogens.* In response to the proposed revised Guidelines for Research Involving Recombinant DNA Molecules published in the **Federal Register** on November 30, 1979 (44 FR 69210), M. T. Goff, Animal and Plant Health Inspection Service, United States Department of Agriculture, has proposed that a classification of plant pathogens be included in the Guidelines. Philip D. Harriman, National Science Foundation, has also submitted a similar proposal. Messrs. Goff and Harriman request that a portion of the Report of a Workshop on Risk Assessment of Agricultural Pathogens, dealing with a hazard classification of plant pathogens, be included in the Guidelines. This Workshop was held on March 20–21, 1978, and the report appeared as Appendix G to the Environmental Impact Assessment which accompanied proposed revised Guidelines published for comment in the **Federal Register** of July 28, 1978 (43 FR 33042). Specifically, they request that the following classification be added to Appendix B of the Guidelines:

> IV. Plant Pathogens
>
> Class 1A Agents—Plant pathogens not in Class 1B.
>
> Class 1B Agents—All organisms that are subject to quarantine restrictions for any of the following reasons:
> (i) Plant pathogens not known to occur in the United States.
> (ii) Plant pathogens that are not widely distributed throughout the ecological range of their hosts in the United States.
> (iii) Plant pathogens subject to U.S. Federal or State eradication or suppression programs.
>
> A USDA permit [2] is required to import or to move plant pathogens across State lines.

Footnote 2 of Appendix B of the proposed revised Guidelines reads as follows:

> USDA permit also required for import and interstate transport.

It is proposed that this footnote be modified to read as follows in order to serve as a single footnote for both animal and plant pathogens:

> [2] A USDA permit, required for import and interstate transport of pathogens, may be obtained form the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

7. *Containment Standards for Large-Scale Research and Production.* A Working Group on Large-Scale Research and Production prepared a draft set of physical containment standards for recombinant DNA research involving more than 10 liters of culture.

Comments on the draft standards were invited through January 10, 1980, in an announcement in the **Federal Register** of November 1, 1979 (44 FR 63074). Preliminary comments were reviewed by the RAC at its December 6–7, 1979 meeting. Comments will be taken into account by the Working Group in its preparation of a revised draft. Revised draft standards will be available from ORDA on February 1, 1980, and will be considered along with comments at the March 6–7, 1980 RAC meeting.

If eventually approved by the NIH Director, the substantive standards for large-scale work could be combined with the procedural standards recommended by the RAC at the September 1979 meeting or a subsequently revised version. The combined standards could then constitute a new Part VII of the NIH Guidelines.

8. *Proposal To Amend Portions of Section III–A–2–a, Viruses of Eukaryotes, of the Proposed Revised Guidelines.* In portions of Section III–A–2–a of the proposed revised Guidelines a level of biological containment requiring the use of "an HV1 host and a vector certified for use in an HV2 system" has been substituted for "an EK1 host and a vector certified for use in an EK2 system." This level of biological containment is abbreviated as "HV1CV" in Table III of the proposed revised Guidelines. At the December 6–7, 1979 meeting, a working group was appointed to review the appropriateness of this level of biological containment for the experiments described in this section of the Guidelines. The working group proposes that HV2 biological containment be required for these experiments. Therefore, the working group proposes that "an HV2 host-vector" be substituted for "an HV1 host and a vector for use in an HV2 system" in Sections III–A–2–a–(1)–(a)–(*2*)–(*c*), III–A–2–a–(1)–(a)–(*3*)–(*b*), III–A–2–a–(1)–(b)–(*1*)–(*b*), III–A–2–a–(1)–(b)–(*2*)–(*b*), III–A–2–a–(2)–(a)–(*1*)–(*b*), III–A–2–a–(2)–(a)–(*2*)–(*b*), and III–A–2–a–(2)–(c)–(*2*)–(*b*). In Table III, "HV2" would be substituted for "HV1CV."

9. *Request for Exemption Under Section I–E–4.* Dr. Francis Macrina of the Virginia Commonwealth University has proposed that *Streptococcus mutans* and *Streptococcus sanguis* be included under the exemption category of Section I–E–4 of the Guidelines on the basis that these bacteria have been shown to exchange chromosomal DNA by known physiological processes. These two organisms would then constitute a new Sublist of Appendix A.

10. *Request for Approval to Clone Exotoxin A Gene of Pseudomonas aeruginosa.* Dr. C. W. Shuster of Case Western Reserve University has requested the RAC to consider whether

the cloning of the exotoxin A gene of *Pseudomonas aeruginosa* in *E. coli* K–12 is permitted. Under exemption I–E–4, *P. aeruginosa* and genus *Escherichia* are listed in Appendix A, Sublist A. However, according to Section I–E, Exemptions:

> It must be emphasized that the following exemptions (4) are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited.

Section I–D–2 prohibits:

> Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (2A) (e.g., botulinum, diphtheria toxins; venoms from insects, snakes, etc).

Dr. Shuster notes that exotoxin A and *P. aeruginosa* are safe under normal laboratory conditions. *P. aeruginosa* is an opportunistic pathogen and exotoxin A is only one of a series of virulence factor necessary to produce pathogenicity. This request was presented to the RAC at its December 6–7, 1979 meeting. At that time, the RAC felt that approval of the proposed experiments appeared to require an exception to a prohibition. Accordingly, the request is being published in the **Federal Register** for a 30 day comment period prior to being reconsidered by the RAC at its March 6–7, 1980 meeting.

11. *Request for Consideration of Appropriate Containment Levels.* Dr. Marvin Schwalb of New Jersey Medical School has requested that the RAC consider the containment levels appropriate to the return of *Schizophyllum commune* DNA cloned in *Saccharomyces cerevisiae* to *Schizophyllum commune.* In addition, Dr. Schwalb requests permission to clone the *S. cerevisiae* derived vector YR414/ura 3 and the *Saccharomyces* 2 plasmid containing yeast or *S. commune* sequences in *S. commune.*

2. *Request for Consideration of Appropriate Containment Levels.* Dr. Olen Yoder of Cornell University has asked the RAC to consider the appropriate containment level for the return of *Helminthosporanium maydis* DNA, which has been cloned in *Saccharomyces cerevisiae*, to the host of origin.

13. *Request to Lower Containment Conditions for Prokaryotic Cloning.* Dr. David Wilson of Cornell University has requested a lowering from P3 to P2 containment levels for experiments involving the cloning of the cellulase gene of *Sporocytophaga ep.* into a thermophilic *Bacillus.*

14. *Recombinant DNA Experiments Involving Foot-And-Mouth Disease Virus.* The RAC at its December 6–7, 1979 meeting recommended, and the Director, NIH, subsequently approved, the cloning of foot-and-mouth disease virus in *E. coli* K–12 at the Plum Island Animal Disease Center. The RAC further recommended, and the Director, NIH, subsequently approved, that prior to the shipment of any clones off of Plum Island, first a working group of the RAC and subsequently the full RAC would examine data arising from the foot-and-mouth disease virus recombinant DNA work on Plum Island.

If any such data are ready for presentation to the RAC, they will be considered at the March 6–7, 1980 meeting. The RAC analysis may include reexamination of the appropriate containment levels for experiments with any clones recommended to be shipped from Plum Island.

15. *Proposed EK2 Host-Vector Systems.* Dr. P. H. Pouwels of the Medisch Biologisch Laboratorium of Rijswijk, The Netherlands, has requested EK2 certification of several *trp* plasmids derived from plasmids pBR345 and pBR313. The *trp* vectors derived from pBR345 carry no antibiotic resistance genes. These plasmids would be used in conjunction with the host x1776 as an EK2 certified host-vector system.

16. *Revision of Section III–C–1–e of the Guidelines.* The proposed revised Guidelines (44 FR 69210), in Appendix C, cite a limited exemption, under Section I–E–5, for certain recombinant DNA experiments in tissue culture:

> Under exemption I–E–5 of these Revised Guidelines are those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus.).

This exemption was promulgated in the **Federal Register** of July 20, 1979 (44 FR 42914). In its original formulation (44 FR 22314), the exemption proposed to include recombinant DNA molecules that contain no more than one-fourth of the genome of a eukaryotic virus. At its December 6–7, 1979 meeting, Dr. Wallace Rowe submitted a document requesting that the RAC reconsider the question of allowing exemption for tissue culture experiments involving recombinant molecules containing no more than one-fourth of the genome of a virus. A working group was appointed to make recommendations for consideration at the March 6–7, 1980, meeting. It has been recommended that Sections III–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e–(1)–(b) of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The proposed revised sections would read as follows:

> III–C–1–e. *All Viral Vectors.*
>
> III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:
>
> *III–C–1–e–(1)–(a).* Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture in the absence of helper virus using P1 containment. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome. For such experiments, no MUA need be submitted but prior notice must be given to the IBC as described in Section III–0 of the Guidelines. The IBC should handle such registration documents as described in Section III–0.
>
> "*III–C–l–e–(1)–(b).* Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.
>
> *III–C–1–e–(1)–(c).* Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild-type strains of the helper virus are able to grow in human cells.

17. *Consideration of Appropriate Containment Levels.* Dr. Charles Jacobs of the University of Texas at Austin has requested permission from the RAC to clone *Wangiella dermatitidis* DNA in *Wangiella dermatitidis* using *Saccharomyuces/E. coli* hybrid plasmids as vectors. Dr. Jacobs requests that the RAC assess appropriate containment conditions.

Dated: January 23, 1980.

Donald S. Fredrickson, M.D.,

Director, National Institutes of Health.

[FR Doc. 80-3041 Filed 1–30–80; 8:45 am]

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

MARCH 6-7, 1980

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

MARCH 6-7, 1980

The Recombinant DNA Advisory Committee (RAC) was convened for its eighteenth meeting at 9 a.m. on March 6, 1980, in Wilson Hall, Building 1, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland. Dr. Jane K. Setlow, (Chairman) Biologist, Brookhaven National Laboratory, presided. In accordance with Public Law 92-463 the meeting was open to the public, except for the review of proposals involving proprietary information as the last item of business on March 7, 1980.

Committee members present for all or part of the meeting were:

Dr. Abdul Karim Ahmed; Dr. David Baltimore; Dr. Kenneth Berns, Dr. Winston Brill; Dr. Francis Broadbent; Dr. Allan Campbell; Mrs. Zelma Cason; Dr. Richard Goldstein; Dr. Susan Gottesman; Dr. Jean Harris; Ms. Patricia King; Dr. Sheldon Krimsky; Dr. Werner Maas; Dr. James Mason; Dr. Elena Nightingale; Dr. David Parkinson; Dr. Samuel Proctor; Mr. Ray Thornton; Dr. LeRoy Walters; Dr. Luther Williams; Dr. Frank Young; Dr. Milton Zaitlin; and Dr. William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. Robert W. McKinney, Enviro Control, Inc., Rockville, Maryland

The following non-voting members and liaison representatives were present:

Dr. Walter R. Dowdle, Center for Disease Control; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Herman Lewis, National Science Foundation; Dr. David Logan, United States Department of Labor; Mr. Melvin Myers, National Institute for Occupational Safety and Health; Dr. Jane Schultz, Veterans Administration; Dr. Sue Tolin, United States Department of Agriculture; and Dr. William J. Walsh, III, Department of State.

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[1] The RAC is advisory to the NIH, and its recommendations should not be considered as final and accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Other National Institutes of Health staff present were:

Dr. Edward Applebaum, NCI; Dr. Stanley Barban, NIAID; Dr. W. Emmett Barkley, ORS; Mrs. Betty Butler, NIAID; Dr. Irving Delappe, NIAID; Dr. John Irwin, ORS; Dr. Micah I. Krichevsky, NIDR; Dr. Richard Krause, NIAID; Mr. Charles Leasure, NIAID; Dr. Malcolm Martin, NIAID; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Dr. Joseph Perpich, OD; Mr. Richard Riseberg, OGC; Dr. Wallace Rowe, NIAID; Dr. Bernard Talbot, OD; and Dr. Rudolf Wanner, ORS.

Others in attendance for all or part of the meeting were:

Dr. Harvey C. Aaron, Occupational Safety and Health Administration; Dr. Queta Bond, National Academy of Sciences; Dr. K. C. Bora, Health & Welfare, Canada; Dr. Peter G. Bostock, New Brunswick Scientific Co., Inc.; Dr, J. Paul Burnett, Eli Lilly & Company; Dr. William Can, Jack Raymond & Company; Mr. Jeffrey Christy, Blue Sheet; Dr. Howard Eddy, Science Council of Canada; Dr. Russel K. Enns, Alpha Therapeutic Corp.; Dr. Stanley Falkow, University of Washington; Dr. Mark Finkelstein, Schering Corporation; Dr. Zsolt Harsanyi, Office of Technology Assessment; Ms. Linda Haverfield, Friends of the Earth; Dr. Paul Hung, Abbott Research Laboratories; Dr. Evelyn Hurlburt, Johns Hopkins School of Hygiene; Dr. I. S. Johnson, Eli Lilly & Company; Dr. Attila I. Kadar, Food and Drug Administration; Dr. Geoffrey Karny, Office of Technology Assessment; Dr. Charles C. Kimble, Food and Drug Administration; Dr. David W. Krogmann, U. S. Department of Agriculture; Dr. Paul Leibowitz, Schering Corporation; Dr. M. A. Levine, Environmental Protection Agency; Dr. F. R. Marks, University of California, San Francisco; Ms. Carmine Masucci, New Brunswick Scientific Co., Inc.; Mr. Bing Miller, New Brunswick Scientific Co., Inc.; Ms. Julie Miller, Science News; Dr. Henry I. Miller, Food and Drug Administration; Mr. J. S. Narrim, Virginia, Office of Governor; Dr. DeLill Nasser, National Science Foundation; Mr. Seth Pauker, National Institute for Occupational Safety and Health; Dr. James D. Punch, UpJohn Co.; Dr. Anthony Robbins, National Institute for Occupational Safety and Health; Dr. Michael Ross, Genentech, Inc.; Dr. Brian Sheehan, Genentech, Inc.; Dr. Richard Silver, Food and Drug Administration; Dr. J. R. Swarz, Staff, United States Senate; Dr. Vicki Weisfeld, Institute of Medicine; Dr. Susan Wright, University of Michigan; and Dr. W. P. Young, Eli Lilly & Company.

I. CALL TO ORDER AND OPENING REMARKS

Dr. Jane Setlow, chairperson, called the meeting to order at 9:00 a.m., March 6, 1980. Dr. Setlow introduced Dr. Kenneth Berns, a new Recombinant DNA Advisory Committee (RAC) member.

Dr. Setlow then called the attention of the RAC to two items, tab 847 and tab 848 which had been included in the material sent to the RAC. She noted that tab 847 is a report of the frequency in nature of Escherichia coli strains that can serve as recipients and/or hosts for genetic information carried by various Escherichia coli recombinant DNA host-vector systems. She said that tab 848 deals with the production of a choriogonadotropin-like factor by a microorganism tentatively named Progenitor cryptocides.

II. MINUTES OF DECEMBER 6-7, 1979 MEETING

The RAC reviewed the minutes of the previous meeting (tab 863). Dr. Walters said he believed there were two substantive errors in the minutes: on page 27, line 32, a semicolon should follow the word "experimental;" on page 31, line 15 should read "host-vector systems are excepted from...."

Dr. Berns noted that on page 15, line 7, he was credited with making a motion concerning an RAC major action. He said he believed Dr. Baltimore actually made this motion.

Dr. Goldstein requested a cross indexing in the discussion of the Foot and Mouth Disease (FMD) proposal beginning on page 4, of the paragraph on page 23 which states that Dr. Krimsky submitted to the RAC material pertinent to the FMD discussion. In addition, he requested that an index of the material supplied by Dr. Krimsky be appended to the minutes.

Mr. Thornton moved approval of the minutes. The minutes, with the changes incorporated, were unaminously approved, with the exception of Dr. Parkinson who abstained.

III. REPORT OF MEETING OF FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Dr. Joseph Perpich, Executive Secretary of the Federal Interagency Advisory Committee (FIC) reported on the FIC meeting (tab 817, 856) of February 27, 1980. He noted that Dr. Maxine Singer, NCI, NIH, had presented a summary of scientific advances in the area of recombinant DNA technology, Dr. William Gartland, ORDA, NIH, had reviewed the recent revisions in the Guidelines, and Dr. Richard Krause, NIAID, NIH, had reported on the NIH recombinant DNA risk assessment program. Dr. Morris Levin had reported on the risk assessment program of the Environmental Protection Agency (EPA). Dr. Bernard Talbot, OD, NIH, had summarized the work of the RAC in reviewing industrial applications, and Dr. Barkley, ORS, NIH, had reviewed the proposed supplement for large-scale physical containment practices. Dr. Burke Zimmerman, OD, NIH, had briefly reviewed Senator Stevenson's bill, S. 2234.

Dr. Perpich said the FIC met primarily to be briefed on RAC activities and to consider what actions, if any, were necessary regarding the industrial application of recombinant DNA technology. Dr. Perpich reported that Dr. Bingham, Assistant Secretary for Occupational Safety and Health, Department of Labor, summarized the interest and involvement of the Occupational Safety and Health Administration (OSHA), and that Dr. Robbins, Director of the National Institute for Occupational Safety and Health (NIOSH), seconded her concerns. In response to these concerns, the Interagency Committee recommended that a subcommittee be created to examine occupational health issues attendant upon industrial application of recombinant DNA techniques. Dr. Perpich said this subcommittee, the Industrial Practices Subcommittee, will be chaired by Dr. Gilbert Omenn of the Office of Science and Technology Policy (OSTP), and will include representatives of the U. S. Department of Agriculture (USDA), the Department of Commerce (DOC), the National Institute for Occupational Safety and Health (NIOSH), the Center for Disease Control (CDC), the Food and Drug Administration (FDA), the National Institutes of Health (NIH), the Occupational Safety and Health Administration (OSHA), the National Science Foundation (NSF), and the Office of Science and Technology Policy (OSTP). Dr. Perpich said the minutes of the FIC meeting would be distributed to the RAC.

Dr. Parkinson stated his belief that OSHA has the authority to regulate the industrial application of recombinant DNA technology and questioned the need for another committee. Dr. Robbins of NIOSH said that OSHA is responsible for regulating workplaces employing recombinant technology as for workplaces using any other technology. He stated that presently there is no clear guidance as to what standards should be used to enforce the OSHA mandate. He said the absence of standards in terms of work practices, engineering, etc, does not eliminate OSHA's responsibility to protect the workplace and that OSHA and NIOSH have begun to examine this area. He added that NIOSH and OSHA do not want to wait a lifetime of worker exposure before examining potential hazards and instituting regulations. He said that if principles of protection are developed early in the industrial application of these techniques, implementation costs to industry would be substantially less than in a "retrofit" situation. Dr. Baltimore stated that regulation of industrial recombinant DNA practices should be part of the general question of regulating the industrial application of microorganisms. Dr. Robbins agreed.

Dr. Parkinson said there are aspects of medical surveillance which should be observed in any workplace where microbiological techniques are employed. Dr. Krimsky asked to what extent OSHA has been involved in the surveillance of this type of workplace. Dr. Parkinson responded that OSHA has not been involved to any great extent. Dr. Robbins pointed out that the notion of developing medical surveillance as part of workplace standards is relatively new.

Dr. Parkinson moved that the RAC request that OSHA begin the formal process of promulgating regulations addressing potential hazards of microbiological techniques in industry, including the innovative use of recombinant DNA techniques. Dr. Ahmed suggested broader language be used to permit greater flexibility. Dr. Parkinson accepted Dr. Ahmed's suggestion.

Mr. Thornton expressed concern over whether the RAC can request OSHA to initiate a particular course of action. He suggested that a request to the Director, NIH would be more appropriate. Dr. Parkinson agreed, and amended his motion to read that the RAC recommends that the Director, NIH, request OSHA to begin the process of initiating possible regulations in the area of the industrial use of microbiological techniques.

Noting that the RAC members had been communicating their concerns directly to the NIOSH and OSHA representatives present at the meeting, Dr. Baltimore stated that he saw no need for the motion and moved to table it. The motion to table was approved by a vote of fourteen in favor, five opposed, and one abstention.

IV. PROCEEDINGS OF THE NIAID WORKING GROUP ON RISK ASSESSMENT

Dr. Krause said that concern had been voiced that, under exceptional circumstances, Escherichia coli K-12 expressing eukaryotic proteins might produce or induce a toxic result. He said two individuals had been selected to assist NIAID in developing a risk assessment workshop to evaluate this concern: Dr. Louis Sherwood, Physician in Chiet and Chairman, Department of Medicine, Michael Reese Hospital, Chicago; and Dr. Philip Paterson, Chairman and Professor, Department of Microbiology and Immunology, Northwestern University. Dr. Krause said that the conference would be held in Pasadena, California, on April 11 and 12, 1980. A general session outlining risk assessment in Escherichia coli K-12 will be followed by concurrent sessions dealing with (1) the hypothetical direct effects of active polypeptides and hormones chaired by Dr. Sherwood, and (2) possible autoimmune responses chaired by Dr. Paterson. Dr. Krause added that the meeting had been publicized and, thus far, a total of 55 people have registered to attend. These include three of the five members of the RAC Risk Assessment Subcommittee, seven non-voting members of the RAC, three members of the Federal Interagency Advisory Committee on Recombinant DNA Research, and representatives of three European nations.

Mr. Thornton requested that the RAC Risk Assessment Subcommittee consider whether other areas of concern exist; e.g., should risk assessment be performed with other host-vector systems. Dr. Zaitlin asked that the Risk Assessment Subcommittee consider the desirability of initiating risk assessment experiments in the plant pathology area.

Dr. Krause then introduced Dr. Stanley Falkow, from the University of Washington, who reported (tab 819, 858) on the unanimous recommendation of an NIAID Risk Assessment Working Group, which met on August 30, 1979, that NIAID not implement risk assessment protocols I and II developed from the proceedings of a Workshop held at Falmouth, Massachusetts, June 20-21, 1977. Protocol I, "Colonization and Transmission of Plasmids by Escherichia coli K-12 in the Gastrointestinal Tract of Humans," and protocol II, "Tests for Transmissibility of Plasmids of Escherichia coli K-12 and χ1776 in Germ-Free Mice" had been developed to evaluate the probability that recombinant DNA, carried by an Escherichia coli K-12 host-vector system, might be transferred to other members of the flora. Dr. Falkow said the Risk Assessment Working Group, after evaluating the data generated by feeding Escherichia coli K-12 to over 60 different people, felt that these protocols would produce negative data. The Working Group, therefore, suggested that a "worst case" type of experiment be substituted for these two protocols. The alternative protocol would utilize instead of strain K-12 a "wild type" Escherichia coli strain, HS, which is known to be nonpathogenic and to colonize the majority of normal individuals who ingest it. The Working Group recommended that the cloning vehicle pBR325, coding for chloramphenicol resistance, be transformed into the HS strain, and that a wild type mobilizable plasmid such as ColE1 carrying chloramphenicol resistance be used as a control. The group felt that this protocol would generate basic scientific information, i.e., mobilization frequencies in the gut, as well as provide risk assessment data for recombinant DNA.

Dr. Falkow said that the NIAID Risk Assessment Working Group had in addition discussed the question of whether Escherichia coli K-12 can be converted into a pathogen. He said that no members of the Working Group, who collectively possess over 20 years of experience in attempting to make K-12 pathogenic, had succeeded in creating a pathogenic K-12. He described some of the attempts.

Dr. Krimsky asked Dr. Falkow why K-12 is difficult to convert into a pathogen. Dr. Falkow stated that K-12, in adapting to the laboratory environment, was modified in major ways including modification in the principal outer membrane proteins. He noted that many Escherichia coli strains isolated from healthy individuals could not, anymore so than K-12, be converted into pathogens by laboratory manipulation. He discussed current epidemiological studies which suggest that a few widely disseminated serotypes of Escherichia coli actually cause most cases of disease.

Dr. Baltimore questioned whether an experiment using the HS strain could be related to recombinant DNA experiments employing K-12 host-vector systems. Dr. Gottesman said that the experiments involving Escherichia coli strain HS would be useful, since baseline numbers for mobilization frequencies in the gut would be generated. Dr. Williams said that he agreed with the recommendations advanced by the NIAID Risk Assessment Working Group.

Dr. Susan Wright from the University of Michigan, asked whether Dr. Stuart Levy of Tufts University had looked for the transfer of plasmids from Bacteriodes to Escherichia coli. Dr. Falkow responded that he did not believe that Dr. Levy had. Dr. Young said he was aware of at least three instances in which such transfer had been demonstrated. Dr. Wright questioned whether the Escherichia coli HS strain was proposed for study because industry wants to use it. Dr. Falkow responded that the HS strain was chosen because it was fed to more human volunteers than any other strain and was known to be harmless. He presumed industry would prefer to work with Escherichia coli K-12 as the organism is so well characterized.

Dr. Williams moved acceptance of the NIAID Risk Assessment Working Group report regarding (1) the recommendations on protocols I, II and III, and (2) the proposal to perform risk assessment experiments with Escherichia coli strain HS. The motion was carried by a vote of twenty in favor, none opposed, and two abstentions.

V. PROPOSED EK2 HOST-VECTOR SYSTEMS

Dr. Campbell began the discussion of tab 820 (843/15). He said that Dr. Pouwels of the Netherlands had requested EK2 and possibly EK3 certification of several plasmids derived from plasmid pBR345. He noted that Dr. Pouwels had not specified the host to be used as part of the host-vector system. Dr. Gottesman said that mobilization data should be supplied by Dr. Pouwels. Dr. Campbell questioned the desirability of certifying new EK2 systems at this time. Dr. Gottesman said that in special cases such EK2 systems might be preferable, as the proposed plasmids do not carry antibiotic resistance markers. She said she thought testing to meet EK3 criteria was unreasonable at the present time. She suggested that before the RAC requests additional information from Dr. Pouwels, the Committee should decide whether certifying a new EK2 system is worthwhile. She moved that the RAC continue certifying EK2 systems. This motion was approved by a vote of twenty in favor, one opposed, and two abstentions. Dr. Gottesman then moved that the proposal be referred to the Plasmid Subcommittee to specify the additional data required of Dr. Pouwels, to request the data, to evaluate it, and to report back to the RAC. The motion passed by a vote of twenty in favor, one opposed, and one abstention.

VI. PROPOSED CLASSIFICATION OF PLANT PATHOGENS

Dr. Brill began discussion of tabs 821, 822 and 843/6. He said that Mr. M. T. Goff, U. S. Department of Agriculture, (USDA) and Dr. Philip D. Harriman, National Science Foundation (NSF) requested the inclusion of a proposed Section IV on plant pathogens within Appendix B of the Guidelines. Dr. Brill said that in his estimation this proposed addition does not provide readers of the Guidelines with useful information. Dr. Zaitlin said that the proposal resulted from an attempt to classify plant pathogens on the basis of risk. A Workshop on Risk Assessment of Agricultural Pathogens on March 20-21, 1978, recommended that plant pathogens should be classified on the basis of whether or not they are quarantined. Dr. Zaitlin said the major thrust of the proposal before the RAC was to instruct investigators unfamiliar with plant pathogens to obtain a permit for transporting the organisms. He agreed that the inclusion of the proposed Section IV is not extremely useful, but said no harm would result from including it in Appendix B. Dr. Zaitlin said that minimally a footnote providing instructions on obtaining a USDA permit should be included in the Guidelines.

Dr. Talbot said that Dr. Fredrickson had considered the inclusion of the proposed Section IV of Appendix B in the 1978 Guidelines but decided against this because the rest of Appendix B is merely a republication of classifications previously promulgated in other published documents whereas this is not, and also because the text of the Guidelines does not refer to such a classification, i.e., nowhere in the Guidelines does it matter whether a plant pathogen is "Class 1A" or "Class 1B."

Dr. Tolin said that some plant pathogens are comparable to CDC Class 5 organisms. She added that USDA would like the NIH Guidelines to indicate that regulations concerning such organisms exist. She stated that USDA recommends approval of the proposal as published in the Federal Register.

Dr. Zaitlin moved that those sections of the Guidelines which refer to plant pathogenic organisms should carry a footnote. The footnote would indicate that a USDA permit is required for import and interstate transport of plant pathogens and tell where permits can be obtained. The proposed footnote published in the Federal Register would be the language used. The motion was unanimously approved by a vote of twenty in favor, none opposed.

VII. PROPOSED AMENDMENT OF SECTION III-O

Dr. Gottesman noted that tab 823 (843/3) is a letter from Dr. Stuart Levy of Tufts University School of Medicine. Dr. Levy suggests that

Section III-O specify the use of poorly mobilizable plasmid vectors. Dr. Gottesman noted that most researchers are currently using poorly mobilizable vectors and that specifying use of these plasmids in the Guidelines would not appreciably change the status quo. She stated that implementing this proposal would raise additional questions. Who would determine whether a given plasmid is poorly mobilizable? What procedures and experiments would be required? As the incorporation of this language into Section III-O would entail several procedural difficulties, Dr. Gottesman recommended that Dr. Levy's proposal not be adopted. She suggested instead that a footnote be included in the Guidelines. Dr. Campbell agreed, saying that the Guidelines encourage researchers to use the host-vector system providing the highest containment when these systems are equally appropriate for the purposes of the experiment.

Dr. Gottesman then moved to reject Dr. Levy's suggestion and instead to add the following footnote to Section III-O.

> "A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed."

The motion was carried by a vote of eleven in favor, none opposed, and five abstentions.

VIII. PROPOSED AMENDMENT OF SECTION III-O

Dr. Baltimore said that tab 824 (843/2) is a letter from Dr. Kent Wilcox of the Medical College of Wisconsin suggesting that the term "eukaryotic protein" in Section III-O is ambiguous. Dr. Wilcox suggests that the relevant section of III-O be amended to read as follows:

> "* * *An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the E. coli K-12 efficiently express as a protein the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.* * *"

Dr. Baltimore said the proposed language is more precise. He questioned the inclusion of "viroids" in this language, but felt that the term could be included for the sake of completeness. He moved acceptance of the proposal as suggested by Dr. Wilcox.

The motion was carried by a vote of eighteen in favor, and none opposed.

IX. PROPOSED AMENDMENT OF SECTION IV-D-2-a

Ms. Cason said that tab 843/1 and 825 is a letter from Mr. David Lester of Princeton, New Jersey, proposing that Section IV-D-2-a, dealing with membership and procedures for the Institutional Biosafety Committee (IBC), be amended so that "non-affiliated members shall be appointed by the governing body of the community in which the institution is located." Ms. Cason said she was unable to support this proposal. While she felt that the public should have some input in the selection process, she questioned the desirability of the community appointing these members. Dr. Talbot noted that two letters had been received during the public comment period, both opposing the proposal.

Dr. Krimsky said that discussion of this issue raises the question of the effectiveness of IBCs. He asked whether IBC function has been evaluated. Dr. Talbot said that a study of IBCs in California has been initiated, and that NIH is considering letting a contract for a larger study, as well as having a meeting of IBC chairmen in the autumn. Dr. Krimsky suggested that the RAC should discuss the issue of assessment of IBCs since IBCs are playing an increasingly important role.

Dr. Ahmed asked whether the current NIH Guidelines stipulate the composition of the local IBC. Dr. Gartland responded that the Guidelines require that at least twenty percent of the IBC membership not be affiliated with the institution. He said the appointment procedure for non-affiliated members differs from institution to institution. He added that the overall composition of the IBC is reviewed by NIH. Dr. Berns said he opposed Mr. Lester's proposal, and raised the question of legal liability. Ms. King noted that legal liability would vary from jurisdiction to jurisdiction and said she opposed mandating what she would consider political representation as opposed to commuity representation. She supported Dr. Krimsky's proposal that the RAC review the functioning of IBCs.

Dr. Krimsky moved to defer consideration of Mr. Lester's proposal until the RAC had a broader discussion of the effectiveness of local IBCs. Dr. Ahmed questioned the mechanism by which this review would occur. Ms. King suggested that the RAC discuss the issue at the June RAC meeting. She commented on a review of Institutional Review Boards (IRB) in which she had participated, and suggested that a copy of the IRB report be distributed to the RAC as reference material.

Dr. Young suggested it would be prudent to wait for the report of the group studying IBCs in California. Dr. Krimsky asked Dr. Gartland for additional information concerning the California study. Dr. Gartland said that Dr. Diana Dutton's group, which is conducting this survey, is supported by NSF's Program in Ethics and Values in Science and Technology. He said they had originally intended to survey all American IBCs, but later restricted the study to California. Ms. King suggested that the RAC invite a representative from the California study to address the RAC at the June meeting. Dr. Gottesman suggested that if NIH holds a meeting of IBC Chairmen in the autumn, RAC members could ask them questions at that time.

Dr. Krimsky moved to defer consideration of Mr. Lester's proposal, to schedule a general discussion of IBCs, and to invite a representative from the group doing the California study to address the RAC at the next meeting. The motion was carried by a vote of sixteen in favor, two opposed, and three abstentions.

X. PROPOSED AMENDMENT OF SECTION ON EUKARYOTIC VIRUS VECTORS

Dr. Baltimore briefly summarized the history of the proposal, tab 826 (843/16). Section I-E-5 permits the Director, NIH, to exempt from the Guidelines experiments which do not present a significant risk to health or the environment. Recombinant DNA molecules, of which no component is derived from a eukaryotic virus, and which are propagated and maintained in cells in tissue culture, have been included under this exemption and are cited in Appendix C of the Guidelines. At the December 1979 RAC meeting, Dr. Wallace Rowe proposed that Appendix C be amended to exempt eukaryotic viral fragments of less than one-quarter of the genome. Dr. Setlow appointed a Working Group to study the proposal. The group arrived at the proposal published in the Federal Register (tab 843/16), which would revise Sections III-C-1-(e), III-C-1-e-1, III-C-1-e-(1)-(a), and III-C-1-e-(1)-(b) of the Guidlines and add a new Section III-C-1-e-(1)-(c). The proposed new Section III-C-1-e-(1)-(a) reads as follows:

> "III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture in the absence of helper virus using P1 containment. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome. For such experiments, no MUA need be submitted but prior notice must be given to the IBC as described in Section III-O of the Guidelines. The IBC should handle such registration documents as described in Section III-O."

Dr. Baltimore said that the notion of a viral Family is well defined. A Family is composed of viruses of a common biochemical type. He said that there is no experimental evidence to date that any eukaryotic virus can dispense with one-third of its genetic information and replicate autonomously or that less than two-thirds of viruses from different Families can fully complement each other's genetic composition.

Dr. Goldstein asked whether the genome of one type of virus could be packaged inside the coat of another virus. Dr. Baltimore replied that transcapsidation between members of two different animal virus Families has never been observed.

Dr. Campbell asked whether low-grade contamination of cells in culture by helper virus is possible. Dr. Dowdle of CDC said that such contamination occurs, generally with viruses being used in the laboratory. Dr. Gottesman questioned whether P1 containment is appropriate for these types of experiments. Dr. Baltimore replied that containment higher than P1 is used in a tissue culture laboratory to prevent contamination of the cell cultures.

Ms. King noted that Dr. Rowe had previously solicited the opinion of several eminent virologists on an earlier version of this proposal, but not on this most recent version. She said she would feel more comfortable if additional comment could be solicited.

Dr. Gottesman then suggested that the RAC vote on each part of the proposal separately. She said the questions which must be addressed when evaluating part one [Sections III-C-1-e, III-C-1-e-(1) and III-C-1-e-(1)-(a) of the proposal] are: (1) Is exemption of two-thirds of a eukaryotic viral genome acceptable in every case; (2) could fragments of viruses of different Families reconstitute to produce a viable virus under these conditions; and (3) what is the probability of adventitious rescue either by cryptic viruses or environmental contaminants. She felt the greatest uncertainty existed concerning the third question and suggested it could perhaps be dealt with either by including a cautioning footnote or by raising containment to P2.

Dr. Baltimore suggested that "in absence of helper virus" be deleted and the following sentence be added to the proposal:

> "For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used."

Dr. Gottesman suggested that the last two sentences of III-C-1-e-(1)-(a) be struck. This would provide for a more conservative review procedure.

Dr. Gottesman moved acceptance of part one of the proposal as amended. Dr. Setlow then called the vote. This part was approved by the RAC by a vote of thirteen in favor, three opposed, and five abstentions.

The motion as passed by the RAC amends the Guidelines as follows:

> "III-C-1-e. All Viral Vectors.
>
> III-C-1-e-(1). Other experiments involving eukaryotic viral vectors can be done as follows:
>
> III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

During the discussion it was also suggested that in Section III-C-1-e-(1)-(a), after the word "Family," reference to footnote 36 be added, and that footnote 36 be revised to make reference to the third report of the International Committee on Taxonomy of Viruses.

Dr. Gottesman began the discussion of parts two [proposed section III-C-1-e-(1)-(b)] and three [proposed section III-C-1-e-(1)-(c)] of the proposal. They read as follows:

> "III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.
>
> III-C-1-e-(1)-(c). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild type strains of the helper virus are able to grow in human cells."

Dr. Gottesman said that these sections pertain to experiments in which helper virus is added to cells in culture. She thought limited possibility for transfer exists in these cases: If there were escape into environment the helper and the recombinant defective virus would probably be diluted away from each other leading to a dead end for replication of the recombinant DNA. She noted that this proposal permits a generalized use of viral vectors beyond that which currently exist in the Guidelines. Dr. Baltimore noted that viruses of CDC Class 3, 4 or 5 could not be used as vectors because of Section I-D-1 of the Guidelines. Dr. Gottesman said that a change in the status of Class 3 organisms will soon be considered by the RAC. Dr. Gottesman questioned whether this proposal should be restricted to CDC Class 1 etiological agents.

She asked whether parts two [III-C-1-e-(1)-(b)] and three [III-C-1-e-(1)-(c)] of the proposal would permit the formation of recombinants between viruses of the same Family. Dr. Baltimore replied that it would, but the proposal requires containment levels taken from the current Guideline levels, i.e., P2 containment for viruses that do not propagate in humans and P3 containment for viruses that do. Dr. Goldstein asked how viruses that "are not able to grow in human cells" are defined. Dr. Baltimore replied that this would be tested by growth in human tissue culture cell lines. Dr. Goldstein asked whether antibodies production in people might constitute a more sensitive testing method, as human viruses which are very difficult or impossible to grow in tissue culture do exist. Dr. Baltimore said that viruses that do not grow in tissue culture would not be used as helper virus.

Ms. King asked whether some mechanism exists through which additional comments on these proposals can be solicited from other virologists. She said she would like to see additional justification before evaluating this proposal. Dr. Krimsky agreed.

Dr. Parkinson proposed to defer discussion of the proposal until more information is provided regarding the possible hazards.

Dr. Williams moved that the Committee defer consideration of part two and three of the proposal [proposed Sections III-C-1-e-(1)-(b) and III-C-1-e-(1)-(c)] until the June meeting and request that a summary be prepared of the data supporting these two Sections. The motion was approved by a vote of thirteen in favor, five opposed, and one abstention.

XI. PROPOSAL TO ELIMINATE SECTION I-D-3

Dr. Brill said that Dr. Clarence Kado of the University of California, Davis (tab 827, 843/4) requested that the RAC delete Section I-D-3 from the Guidelines. Section I-D-3 prohibits the deliberate creation of plant pathogens with increased virulence and host range beyond that which occurs by natural genetic exchange. Dr. Brill said that defining virulence and host range is very difficult. He said that virulence can be increased by non-recombinant techniques in the laboratory, but that it is far more difficult to increase the virulence of an organism which can then compete in nature. He pointed out that the use of recombinant technology in plant pathology would facilitate important studies of the basis of pathogenesis. Dr. Zaitlin concurred.

Dr. Krimsky asked whether new information had been advanced to cause plant pathologists to reexamine their ideas on this prohibition. Dr. Zaitlin responded that a workshop had been held in 1978, and the concensus of the participants was that no plant pathogen would be created by recombinant DNA techniques which would be more dangerous than the wild type organisms themselves. Dr. Tolin said that Section I-D-3 has discouraged progress in this area of research.

Dr. Gottesman said that she saw no reason to continue to prohibit the study of plant pathogens. However, she argued against deleting Section I-D-3 without considering the ramifications on other sections of the Guidelines. She suggested that plant pathogens might be removed from the prohibitions by the same mechanism that will be proposed for CDC Class 3 pathogens. She said the proposal she would be presenting later in the meeting for Class 3 organisms would remove these organisms from the prohibited category and lower review procedures one notch, i.e., experiments involving Class 3 organisms would require prior review by the RAC but would not require prior notice in the *Federal Register*.

Dr. Gottesman moved that the RAC take no action at this time. Prior to the next meeting a proposal would be published in the *Federal Register* to delete Section I-D-3 and to consider plant pathogens along the same model to be developed for CDC Class 3 agents.

The motion was accepted by a vote of nine in favor, six opposed, and three abstentions.

XII. PROPOSAL TO INCLUDE ALL SPECIES OF GENUS ERWINIA IN APPENDIX A, SUBLIST A

Dr. Brill presented the request (tab 827, 843/5) from Dr. Clarence Kado of the University of California, Davis to include all *Erwinia* species in Sublist A of Appendix A of the Guidelines. Currently, only one *Erwinia* species, *Erwinia amylovora*, is included on this list of species that exchange DNA by known physiological processes. Dr. Brill said that all the *Enterobacteriaceae*, which include *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Erwinia* are very closely related. He noted that gene transfers between the *Enterobacteriaceae* occur frequently. He moved that Dr. Kado's request be approved and that all species of *Erwinia* be included in Sublist A, Appendix A.

Dr. Gottesman recounted some of the history of the development of Appendix A, and summarized the types of exchanges the RAC previously felt should be demonstrated before an organism is included in Appendix A. Dr. Fredrickson in his Decision Document of December 22, 1978 had accepted as criteria for inclusion in Appendix A,

> "Organisms which exchange chromosomal genetic information which becomes stably integrated into the host chromosome" and
>
> "Organisms which exchange chromosomal information that is not necessarily integrated into the chromosome of the recipient (for instance, transfer via F' or R);"

He did not at that time, however, accept

> "Organisms which show evidence of a plausible mechanism for exchange (e.g., R' formation or evidence of mobilization of chromosomal genes by an Inc P-1 plasmid)" or
>
> "Organisms which can receive or donate broad host range plasmids."

She questioned whether Dr. Kado's argument of relatedness by 20 percent sequence homology was sufficient to warrant the inclusion of the genus Erwinia in Appendix A.

Dr. Falkow recounted his participation in the development of Appendix A. He said that he believes that chromosomal sequence homology of 20 percent or greater indicates sufficient relatedness to warrant inclusion of organisms on sublists in Appendix A. He said that Erwinia shares a sufficient core of sequence homology with the other Enterobacteriaceae to warrant inclusion in Appendix A, Sublist A. Dr. Young agreed.

Dr. Brill restated his motion to change the listing on Sublist A, Appendix A from Erwinia amylovora to the genus Erwinia. The RAC accepted this recommendation by a vote of fourteen in favor, none opposed, and three abstentions.

XIII. PROPOSAL TO AMEND PORTIONS OF SECTION III-A-2-a

Dr. Nightingale said that she and Dr. Brill had been asked to examine the applicability of the terminology "HV1CV" to host-vector systems other than Escherichia coli K-12. She said a recommendation (tab 843/8) to change the biological containment requirement from HV1CV to HV2 within the subsections of Section III-A-2-a was advanced since it was felt that the CV terminology could not be generalized to host-vector systems other than Escherichia coli K-12. She said that in the EK system, CV containment depends on the plasmid, and the properties of these plasmids can not be generalized to other host-vector systems. She added that in proposing this change she hoped to encourage discussion of the issue. She moved that the proposal be approved as it appeared in the Federal Register.

Dr. Gottesman supported the contention that EK1CV could not be generalized to other host-vector systems. She said that historically the EK1CV nomenclature stood for an EK1 host and a vector certified for use in an EK2 system, and was a position between EK1 and EK2 containment. Dr. Baltimore said the CV nomenclature is meaningless in the yeast system and should be amended. He said HV2 containment is too stringent and suggested that HV1 be substituted for HV1CV. Dr. Gottesman said a proposal substituting HV1 containment for HV1CV would have to be republished in the Federal Register as a proposed major action.

The motion to amend the HV1CV nomenclature of the Guidelines to HV2 was denied by a vote of seven in favor, eleven opposed, and three abstentions.

A proposal to change the nomenclature from HV1CV to HV1 will appear in the *Federal Register* prior to the June RAC meeting.

XIV. PROPOSED EXEMPTION FOR STREPTOCOCCUS MUTANS AND STREPTOCOCCUS SANGUIS

Dr. Gottesman introduced the proposal of Dr. Francis Macrina of the Virginia Commonwealth University to include *Streptococcus mutans* and *Streptococcus sanguis* under the exemption category of Section I-E-4 of the Guidelines (tab 829, 843/9). She said this proposal requests the institution of a new sublist in Appendix A. She said it is a unique proposal in that Dr. Macrina proposes a one-way exemption. Dr. Campbell noted that Dr. Fredrickson, in the decision document accompanying the 1978 Guidelines, specifically endorsed one-way transfer lists in Appendix A.

Dr. Gottesman said the data supplied by Dr. Macrina demonstrate the transformation of *Streptococcus sanguis* by *Streptococcus mutans* DNA, but exchange from *Streptococcus sanguis* into *Streptococcus mutans* has not been demonstrated. *Streptococcus mutans* to date appears to be nontransformable. Dr. Gottesman said that the data showing unidirectional transfer is good, transformation occurs at reasonable frequencies and apparently occurs *in vivo*. Dr. Ahmed asked whether chromosomal gene transfer occurred. Dr. Gottesman said that it did. Dr. Gottesman moved that a new sublist of Appendix A be established for one-way transfer from *Streptococcus mutans* to *Streptococcus sanguis*.

The RAC unaminously recommended acceptance of the motion by a vote of nineteen in favor, and none opposed.

XV. PROPOSALS TO CLONE EXOTOXIN A PROTEIN OF PSEUDOMONAS AERUGINOSA

Dr. Broadbent introduced a request (tab 830, 843/10) from Dr. C. W. Shuster of Case Western Reserve University to permit the cloning of the *Pseudomonas aeruginosa* exotoxin A gene in *Escherichia coli* K-12. Dr. Broadbent noted that this item had been considered at the December 6-7, 1979 RAC meeting. He said the RAC at that time recommended that the proposal be published in the *Federal Register*. In the period between the December and March RAC meetings, Drs. James Miller of the University of Louisville and Dr. Stanley Falkow of the University of Washington submitted similar proposals. In support of his request, Dr. Stanley Falkow submitted extensive documentation concerning *Pseudomonas aeruginosa*. Dr. Broadbent said Dr. Falkow argued that exotoxin A is not a potent toxin in the class of botulinum or diptheria toxin and is not a primary determinant of disease.

Exotoxin A thus should not be included under the I-D-2 prohibition. Dr. Broadbent said he found Dr. Falkow's argument persuasive. Dr. Young agreed. He said that noncompromised individuals can be colonized by Pseudomonas aeruginosa without mortality. He added that Pseudomonas aeruginosa is a relatively nonpathogenic organism unlike Corynebacterium diptheriae or Clostridium botulinum in which a single toxin is the determinant of disease. Dr. Falkow said that while exotoxin A is an important virulence determinant of Pseudomonas aeruginosa, the symptoms experienced by infected individuals are not directly related to the toxin per se.

Dr. Campbell asked what degree of containment was used in working with Pseudomonas aeruginosa. Dr. Falkow replied that Pseudomonas aeruginosa is a CDC Class 1 agent.

Dr. Gottesman suggested the RAC first ascertain whether exotoxin A is a "toxin potent for vertebrates" in the context of the Guidelines. Mr. Thornton agreed; he added that the word "potent" should be defined. Dr. Falkow said that exotoxin A is several hundred fold less potent than botulinum and one-fiftieth as potent as diptheria toxin.

Dr. Young moved that exotoxin A, under the Guidelines, not be considered a potent toxin similar to botulinum toxin. Dr. Campbell noted that the effect of this motion would be to permit exotoxin A to be treated as any other gene of Pseudomonas aeruginosa. He said exotoxin A could thus be cloned into any organism on Sublist A, Appendix A, as an exempt experiment. The RAC accepted the motion by a vote of seven in favor, two opposed, and ten abstentions.

Mr. Thornton suggested it would have been more appropriate to approve the cloning of the exotoxin A gene in Escherichia coli K-12 as requested, rather than approving the broad motion passed. Dr. Campbell agreed.

Dr. Young moved to reconsider the former motion. Dr. Mason agreed, stating that the general issue of what constitutes a potent toxin under the Guidelines should be examined. The motion to reconsider the previous motion was approved by a vote of fifteen in favor, none opposed, and two abstentions.

Dr. Young then made a motion to approve the cloning of the Pseudomonas aeruginosa exotoxin A gene in Escherichia coli K-12, under P1 + EK1 conditions. The motion passed by a vote of fourteen in favor, none opposed, and three abstentions.

Dr. Setlow later appointed Drs. Broadbent, Maas, and Mason to examine the question of potency of a toxin under the Guidelines.

XVI. PROPOSALS REQUIRING ASSIGNMENT OF CONTAINMENT LEVEL

A. Request to consider containment appropriate to returning *Helminthosporium maydis* DNA cloned in yeast to the host of origin

Dr. Zaitlin introduced the request (tab 833, 843/12) of Dr. Olen Yoder of Cornell University to consider the containment level appropriate for the return of *Helminthosporium maydis* DNA, which had been cloned in *Saccharomyces cerevisiae,* to the host of origin. Dr. Zaitlin said that there are a number of races of the fungus *Helminthosporium maydis,* one of which, race T, produces a potent toxin. Certain varieties of corn are susceptible to this fungus. Dr. Zaitlin said that Dr. Yoder intends to study the non-toxin producing race O which is not a pathogen in the U. S. except for the very southern tip of Florida. Dr. Zaitlin recommended approval of the project. Dr. Brill seconded the motion.

Dr. Gottesman asked if *Helminthosporium maydis* might exchange genetic information with other organisms. Dr. Zaitlin responded that *Helminthosporium maydis* is a difficult organism to grow and genetic exchange has not been studied.

Dr. Gottesman noted that this experiment returns cloned DNA to the host of origin. She stated that the RAC at some point might wish to consider the generic issue of return of DNA cloned in yeast host-vector systems to the host of origin. Dr. Gottesman said that this issue is complicated by the variety of yeast cloning vehicles, and their behavior in yeast. She said the yeast vector Dr. Yoder proposes to employ recombines into the chromosome. Random pieces of yeast chromosome thus may be picked up by the integrated plasmid. However, she said she felt the proposed experiment to be acceptable at the P2 level requested by Dr. Yoder.

Dr. Goldstein requested that Dr. Zaitlin summarize information about the pathogenicity of this organism. Dr. Zaitlin responded that race T of *Helminthosporium maydis* is a serious corn pathogen in the United States. He said that race O is not. He pointed out that Dr. Yoder does not propose any experiments with plants, but wishes to study race O under laboratory conditions. Dr. Gottesman asked whether race O ever produces a toxin. Dr. Zaitlin said it apparently does not produce a toxin. Dr. Goldstein asked how race O induces disease. Dr. Tolin responded that race O causes a mild disease by direct action on the leaves in contrast to race T, the toxin of which causes complete collapse of the leaf.

Dr. Goldstein asked whether the lack of health surveillance at Cornell University for experiments conducted at the P1 or P2 levels of containment conforms with NIH policy. Dr. Gartland said the Guidelines mandat that the institution determine the necessity for health surveillance but do not mandate any required health surveillance program, and that health surveillance varies from university to university.

Dr. Brill moved acceptance of Dr. Yoder's request at the P2 level of physical containment. Dr. Zaitlin absented himself during the vote. The RAC recommended approval of the request at the P2 level of containment by a vote of eight in favor, none opposed, and nine abstentions, including Dr. Zaitlin.

B. Request to evaluate containment appropriate to returning *Schizophyllum commune* DNA cloned in yeast to the host of origin

Dr. Broadbent said that tab 831 (843/11) concerns a request from Dr. Marvin Schwalb of the New Jersey Medical School to consider containment levels appropriate for the return of *Schizophyllum commune* DNA cloned in *Saccharomyces cerevisiae* to *Schizophyllum commune*. Dr. Broadbent said Dr. Schwalb also requests permission to clone the *Saccharomyces cerevisiae* derived vector YR414/ura 3 or the *Saccharomyces cerevisiae* 2 mμ plasmid containing *Saccharomyces cerevisiae* or *Schizophyllum commune* sequences in *Schizophyllum commune*. He said Dr. Schwalb is proposing to use P2 physical containment levels. Dr. Broadbent said that *Schizophyllum commune* is a non-pathogenic basidiomycete which is widespread in nature. He said *Schizophyllum commune* does not form asexual spores, and forms spores only under easily controllable genetic and environmental conditions. He recommended approval at the P2 level of containment. Dr. Williams and Young concurred, noting that the organism is well controlled under laboratory conditions and that asexual sporulation does not occur.

Dr. Walters pointed out that in a letter of December 13, 1979, Dr. Schwalb suggests that *Schizophyllum commune* be considered as an HV1 system. Drs. Young and Gottesman said that approval for the specific experiment is being considered, and HV1 certification of *Schizophyllum commune* is not under consideration.

Dr. Broadbent moved approval of the proposed experiments at P2 physical containment. The motion passed by a vote of eighteen in favor, none opposed, and one abstention.

C. Consideration of appropriate containment for cloning *Wangiella dermatitidis* DNA

Dr. Broadbent presented the proposal of Dr. Charles Jacobs of the University of Texas at Austin (tab 832, 860, 843/17). He said Dr. Jacobs requests permission to clone *Wangiella dermatitidis* DNA in *Wangiella dermatitidis* using a *Saccharomyces cerevisiae*/*Escherichia coli* hybrid plasmid as vector.

Dr. Broadbent said that Wangiella dermatitidis is normally a saprophytic soil organism. In some instances Wangiella dermatitidis is pathogenic and can cause a deep mycosis in humans. He noted that the Center for Disease Control (tab 860) suggests that Wangiella dermatitidis should be handled as a Class 2 agent. Dr. Broadbent recommended P3 containment.

Dr. Mason said the organism can infect normal uncompromised individuals. He said Wangiella dermatitidis has a propensity to invade the central nervous system.

Dr. Nightingale asked if the infection can be treated. Dr. Mason said treatment is difficult and the drugs used are highly toxic. Dr. Young said that he did not believe the proposed recombinant DNA experiment was a hazard, but he was concerned that in dealing with the organism itself deep mycoses might be contracted. He suggested P3 containment.

Noting that Dr. Jacobs proposes to select cycloheximide resistant transformants, Dr. Gottesman asked if cycloheximide would ever be used to treat a Wangiella dermatitidis infection. Dr. Young said it would not.

Dr. Campbell asked if other yeast genes might be linked to the cycloheximide resistance gene. He asked how resistance might be expressed. Dr. Baltimore noted that alterations in membrane permeability are common mechanisms of antimicrobial resistance. Dr. Nightingale asked whether an alteration in membrane permeability might compromise treatment with drugs used clinically to treat the disease. Dr. Gottesman requested that the investigator assess potential alterations in therapeutic drug sensitivity resulting from the introduction of cycloheximide resistance.

Dr. Mason moved to approve the request at P3. Dr. Baltimore asked which plasmid vectors were to be used. Dr. Walters suggested that the investigator be telephoned. Dr. Setlow agreed and postponed the discussion until further information could be obtained.

Following a telephone conversation with Dr. Jacobs, Dr. Milewski reported to the RAC that Dr. Jacobs had agreed to utilize one of the HV2 certified Saccharomyces cerevisiae/Escherichia coli hybrid plasmid vectors and not to select for cycloheximide resistance. Dr. Mason moved approval at the P3 level of containment. The RAC approved the motion by a vote of ten in favor, none opposed, and four abstentions.

D. Request to lower containment for experiments involving a thermophilic Bacillis and the cellulase gene of Sporocytophaga

Dr. Williams introduced a proposal (tab 834, 852, and 843/13) from Dr. David Wilson of Cornell University. He said Dr. Wilson would like to use a plasmid isolated from Bacillus stearothermophilus or from other thermophilic Bacilli to transfer the cellulase gene from the thermophilic organism Sporocytophaga into a thermophilic Bacillus. Dr. Williams said Dr. Wilson seeks a reduction in containment level from P3 to P2.

Dr. Williams said Dr. Wilson advances two arguments in support of his request:

(1) Both of the organisms are thermophilic. The optimum temperature for growth of thermophilic organisms is 65 degrees.

(2) It appears the organisms have a symbiotic relationship.

Dr. Williams said he found the proposal deficient. He said that additional information on the plasmid and the organisms to be employed was required. He moved denial of the request until such documentation is provided. Dr. Young seconded the motion.

Dr. Campbell said that the P3 level for experiments involving nonpathogenic prokaryotes (Section III-B-3 of the Guidelines) was set as an upper limit and not because such experiments were all judged to really require that containment. When data on individual cases indicates a lower containment is appropriate, the level should be lowered.

Dr. Brill said that enzymes of thermophiles almost always function optimally at high temperatures and poorly at 37 degrees C. Dr. Goldstein asked if thermophilic enzymes have no activity at 37 degrees C. Dr. Brill replied that he was not certain activity would be zero at 37 degrees C. Dr. Gottesman said that the extent of symbiosis is not clear; she said the Bacillus apparently grows without the Sporocytophaga in certain circumstances.

Dr. Setlow called the question. Dr. Zaitlin left the room during the vote as Dr. Wilson is affiliated with Cornell University. The RAC denied the request to lower containment from P3 to P2 by a vote of twelve in favor, four opposed, and three abstentions. The RAC requested that additional information be supplied by the investigator.

E. Request to clone the DNA of Schistosoma mansoni

Dr. Maas introduced a proposal (tab 853) from Dr. S. B. Henriques of Brazil to clone the DNA of Schistosoma mansoni. Dr. Maas said the use of recombinant technology to study this organism may result in the production of a vaccine against the parasite. He said Schistosoma mansoni is listed as a CDC Class 3 agent. Dr. Talbot pointed out that under the current Guidelines, a request to study a Class 3 agent must be published in the Federal Register as a proposed major action.

Action on Dr. Henriques' request would have to be deferred until the June meeting.

Dr. Gottesman and Ms. King questioned why an investigator in Brazil would desire an interpretation from the RAC. Dr. Maas suggested that many Brazilian investigators were trained in the United States and would prefer to follow the NIH Guidelines. Dr. Young said schistosomiasis is an important disease affecting millions of individuals in tropical or subtropical regions. He believed the RAC should be responsive to this type of query. Dr. Setlow suggested that Dr. Henriques be queried as to his reasons for submitting this request to the RAC. If appropriate, the issue will be discussed at the June RAC meeting following publication in the Federal Register.

F. Request to construct a plasmid bank for use in Anacystis and Escherichia coli

Dr. Brill introduced a letter (tab 854) from Dr. Louis Sherman of the University of Missouri concerning a project initiated during a sabbatical in Holland.

Dr. Brill said that Dr. Sherman is studying a unicellular blue-green algae, Anacystis, and has successfully transformed this organism with indigenous plasmids as well as with Escherichia coli plasmids. Dr. Sherman intends to construct a plasmid hybrid to be transferred between Escherichia coli and Anacystis. Dr. Brill said that an MUA describing this work had been submitted and that containment levels of P2 + EK2 were indicated in the MUA. He said Dr. Sherman now requests a lowering of containment. Dr. Brill recommended approval of the request at the P1 level of containment.

Dr. Gottesman offered her interpretation of these experiments. She said the transformation experiments into Escherichia coli K-12 which Dr. Sherman described may be performed at P1 + EK0. She said that more complicated experiments would require closer scrutiny. Dr. Brill agreed that Dr. Sherman does not provide a clear idea of future experiments. Dr. Talbot suggested that the investigator be informed by ORDA of what he can do under the Guidelines. Should he desire to do experiments beyond those permitted by the Guidelines, ORDA would suggest he submit details for publication in the Federal Register prior to discussion by the RAC. The members of the committee concurred.

G. Request for an exception to a prohibition in order to study Cauliflower Mosaic Virus aphid transmissibility

Dr. Zaitlin introduced the proposal (tab 857) from Dr. Robert Shepherd of the University of California, Davis. Dr. Zaitlin said he believed this request should be published in the Federal Register as a proposed major action and could not be acted on at this meeting.

Dr. Zaitlin said Dr. Shepherd would like to study that portion of the Cauliflower Mosaic Virus genome which confers aphid transmissibility. Dr. Zaitlin said the Guidelines contain a prohibition against using aphid transmissible strains of Cauliflower Mosaic Virus as recombinant DNA vectors. Dr. Zaitlin said that this stricture against using aphid transmissible Cauliflower Mosaic Virus is inconsistent, as there is no similar prohibition for other viruses.

Dr. Zaitlin said Dr. Shepherd also requests permission to insert that portion of the genome which confers aphid transmissibility into non-aphid transmissible strains. These experiments would increase the virulence or host-range of a pathogen. Dr. Zaitlin said that Cauliflower Mosaic Virus has a very restricted host-range. It attacks certain members of the cabbage Family. In addition, the virus does not replicate in the aphid and thus does not persist in the aphid. He noted that insect-proof cages are used in these studies.

It was agreed that this proposal would appear in a Federal Register announcement as a proposed major action (dealing with both the specific and the generic case) and be discussed at the June RAC meeting.

H. Introduction of rat insulin gene into mouse embryos

Dr. Berns summarized a proposal from Dr. Howard Goodman of the University of California, San Francisco. He said Dr. Goodman proposes to use a defective SV40 genome as a vector to introduce the genes for rat (or human) insulin (or growth hormone) into mouse embryonic cells. The vector will hopefully integrate and rat insulin will be expressed. Dr. Berns said the two cell stage embryos would then be transplanted into a pseudopregnant surrogate mother mouse. These embryos should develop into adult mice producing chemically distinguishable rat insulin. Dr. Berns said Dr. Goodman in his cover letter suggests that this type of experiment could be covered by Section III-C-7-(a) of the Guidelines. In the text of the proposal, however Section III-C-1-(b)-(2) is cited. After further discussion and consideration of these two sections of the Guidelines, a concensus developed among the Committee members that Section III-C-1-(b)-(2) covers this experiment.

XVII. PROPOSAL TO REMOVE CDC CLASS 3 ORGANISMS FROM PROHIBITED EXPERIMENTS

Noting that this proposal must be published in the Federal Register for comment, Dr. Gottesman presented a draft proposal to remove CDC Class 3 organisms from Section I-D-1 of the Guidelines. She said that a working group had been appointed to review the desirability and the possible mechanisms for removing Class 3 agents from the prohibited category.

After some discussion, the working group agreed that Class 3 etiological agents should be removed from the prohibited category. The approach the working group recommends would require RAC review of proposals but would not require prior publication of the proposals in the Federal Register for a comment period.

Dr. Zaitlin said experiments which increase the virulence and host range of plant pathogens currently prohibited by Section I-D-3 could effectively be dealt with under this proposal. Dr. Gottesman agreed to include language concerning plant pathogens in the proposal.

Dr. Krimsky asked about the current status of the pending revision of the CDC classification of etiological agents. Dr. Krause replied that the release date is unknown. Dr. Talbot said that the NIH Guidelines refer specifically to the 1974 CDC Classification of Etiological Agents and that therefore the 1974 edition would be used for the Guidelines until the RAC recommends otherwise.

Dr. Gottesman asked whether the draft language should specify that all work with Class 3 agents would require RAC prior review or should automatically allow it at the P3 level of containment with a lower level possible after RAC review. Dr. Nightingale asked if the number of reviews would be reduced by the inclusion of the P3 containment provision. Dr. Young replied that most facilities studying Class 3 pathogens are equipped with a P3 facility, and he would expect inclusion of the P3 containment level clause to decrease the RAC's burden.

Dr. Walters asked if certain Class 3 agents might be of particular concern. It was noted during this discussion that Smallpox, Whitepox and Alastrim are included among Class 3 agents. Dr. Young and Dr. Berns suggested that mention of these etiological agents in the Guidelines be flagged to indicate that due to World Health Organization efforts at eradication, all activities, including storage of these agents, are restricted to a single national facility.

Dr. Gottesman asked whether the introduction of genomic fragments from Class 3 agents into eukaryotic viral systems might pose any concern. Dr. Berns replied that if less than the whole viral genome is being cloned no special problems should arise.

A proposal covering Class 3 agents will be published in the Federal Register for comment prior to the June meeting, and will be reconsidered then.

XVIII. PROPOSAL ON EQUIVALENCY OF HV SYSTEMS WITH EK SYSTEMS

Dr. Williams presented a proposal submitted by Dr. Richard Novick (tab 828), who was not present at the meeting. He said Dr. Novick has attempted to address the question of whether the Guidelines should be amended to institute complete equivalency between EK and HV host-vector systems. Dr. Williams said he believed Dr. Novick prefers equivalency between the EK systems and the three HV systems certified to date only in shotgun cloning experiments. Dr. Talbot and Dr. Gottesman said they found Dr. Novick's proposal confusing.

Dr. Gottesman cited two major issues on the question of equivalency: (1) Should equivalency be extended to return to host of origin experiments using HV systems; and (2) Should IBCs be authorized to lower containment levels for characterized clones in HV systems, as they were previously permitted to do for EK systems.

Dr. Gottesman suggested that equivalency should not be extended to return to host of origin experiments, i.e., Sections of the guidelines (such as III-C-6) which permit return to host of origin of DNA propagated in Escherichia coli K-12 should not be expanded to also include propagation in other HV systems. She said the characteristics of the certified hosts and vectors vary from system to system, and the general principle should not be applied to all cases. For example, she noted that a wide range of Saccharomyces cerevisiae plasmids exist, some of which carry information which might have an effect on other organisms. In addition, certain yeast plasmids can integrate into the chromosome. She said that for these reasons she views the yeast host-vector systems as more complex than the Escherichia coli systems. She moved that the Guidelines should not be amended to extend equivalency to return to host of origin experiments; rather, each case should be evaluated individually. A straw vote was taken to determine the sentiment of the RAC on this issue.
Dr. Gottesman's proposal was supported by a vote of nine in favor, none opposed, and five abstentions.

A straw vote was then taken to ascertain the sentiment of the RAC on permitting local IBCs to lower containment for characterized clones. The RAC supported this proposal by a vote of nine in favor, one opposed, and three abstentions. A working group composed of Drs. Novick, Brill, Campbell and Gottesman was appointed to develop language for publication in the Federal Register prior to the next meeting.

XIX. PROPOSAL TO INCLUDE SACCHAROMYCES CEREVISIAE UNDER SECTION III-O OF THE GUIDELINES

Dr. Setlow said she had drafted a proposal (tab 855) to include *Saccharomyces cerevisiae* laboratory strains under Section III-O of the Guidelines. She said additional information concerning yeast has been obtained, including information that *Saccharomyces cerevisiae* is unable to express higher eukaryotic genes. She said she believed that some of the arguments advanced earlier in support of HV1 certification for *Saccharomyces cerevisiae* could also be advanced to support the inclusion of *Saccharomyces cerevisiae* under Section III-O. She asked for comments concerning this proposal.

Dr. Gottesman said that the basic characteristics of the *Saccharomyces cerevisiae* HV systems should be reviewed by the RAC. Dr. Baltimore said that laboratory *Saccharomyces cerevisiae* strains are as enfeebled as *Escherichia coli* K-12.

Dr. Gottesman questioned whether the stipulation in Section III-O that any deliberate attempt to express a eukaryotic protein product must be reviewed by the local IBC is sufficient review for experiments using yeast HV systems. Dr. Goldstein said the implications of an *Saccharomyces cerevisiae* system expressing eukaryotic proteins should be examined in greater depth.

Dr. Young suggested that the data indicating that *Saccharomyces cerevisiae* systems do not express higher eukaryotic proteins be published in the *Recombinant DNA Technical Bulletin*. Dr. Goldstein agreed. Dr. Setlow said she would ask the investigators if they would publish this data in the *Bulletin*. Proposed language including *Saccharomyces cerevisiae* under Section III-O of the Guidelines will be published in the *Federal Register* prior to the next meeting.

XX. REPORT OF VISIT TO ELI LILLY AND COMPANY

Dr. Walters reported on the visit of January 28, 1980, which he, Dr. Emmett Barkley, Mr. Ray Thornton, and Dr. Robert McKinney had made to the Eli Lilly plant in Indianapolis, Indiana. Dr. Parkinson asked about Dr. McKinney's background. Dr. McKinney said that he has consulted for the NIH Office of Research Safety for four and one-half years. He possesses a degree in epidemiology with a virology specialty. He had worked at the bench for over 20 years, including 10 years at Fort Detrick where he was a member of the Safety Committee, and had much experience in the design of biological containment facilities.

Dr. Walters said the visit had been arranged by Dr. Barkley at Dr. Fredrickson's request. He said the visit had two goals: (1) to examine the containment facilities at Lilly, and (2) to gather information that would be helpful in revising the draft large-scale standards.

Dr. Walters said the visit consisted of (1) a discussion of review, monitoring, and health surveillance procedures, (2) an inspection of several laboratories and a factory facility, and (3) a discussion of the November 14, 1979, draft large-scale standards.

Dr. Walters reported that the group had seen fermentors of three different sizes: 10-liters, 150-liters and fermentors in the range of 2,000 to 50,000 gallons. He said several potential containment problems must be confronted when dealing with standard fermentors. These include: (1) leakage or spills of inocula introduced or of samples removed from the fermentor for testing, (2) exhausting of aerosols produced during fermentation, (3) leakage of aerosols around the fermentor agitator blade shaft, and (4) the vulnerability of the pipe and valve at the bottom of the vessel.

Mr. Thornton said he wished to add three additional points to Dr. Walters' report. He said Eli Lilly engineers told the group that: (1) most of the supply lines to the facility which contains two 150-liter fermentors were independent of supply lines to the rest of the plant with the exception of electric power and steam lines which were held in common with the rest of the plant, (2) a negative pressure differential within the two 150-liter fermentors ensured that any leakage around the shafts would be siphoned into the fermentors and (3) there were no drain valves at the bottom of these 150-liter units.

Dr. Barkley agreed with the reports of Dr. Walters and Mr. Thornton, and said the group had concluded that the engineering designs employed were appropriate.

Dr. Parkinson asked if the plant was organized. Dr. Johnson replied that the plant is not unionized. Dr. Parkinson asked if members of the group had had the opportunity to speak with workers in the absence of management. Dr. McKinney said he had spoken with two or three people in the absence of management and was very satisfied with their responses to his questions. Dr. Baltimore asked whether the group had spoken with production line workers not necessarily using recombinant methods. Mr. Thornton replied that the group had had the opportunity, but he did not know if anyone had availed themselves of it. Dr. Parkinson asked whether workers are represented on the Health and Safety Committee in the plant, and if the group had spoken with these members. Dr. Johnson replied that there are worker representatives on the Committee, and Dr. McKinney said that the visitors had not spoken with them. Dr. Mason asked about the Eli Lilly employee health and medical surveillance program. Dr. Walters, Barkley, and Johnson described the program including annual physical examination and serum collection.

Dr. Parkinson asked if any environmental monitoring procedures had been implemented in the plant. Mr. Young replied that during operation of the 150-liter fermentor, a monitoring program measures both the exhaust gases of the fermentors and the room environment twice a week. He said these operations were initiated in October 1979, and no recombinant organisms have been found to date. Dr. Goldstein asked how frequently the fermentors are used. Mr. Young replied that these fermentors were cycled at the rate of four harvests per week. Dr. Young asked if either concentrated air samples or plate samples are examined in the survey. Mr. Young said both types of collection were used. Dr. Baltimore asked how negative pressure in the fermentors was maintained. Mr. Young replied that a vacuum source and a built-in sensor control unit maintain negative pressure.

Dr. Parkinson asked whether worker education manuals dealing with emergencies or with other potential health-related hazards are available in the plant. Dr. Walters said emergency procedures are specified in the documentation provided to the group by Eli Lilly. In addition, a detailed manual for recombinant DNA projects, including emergency procedures, was available.

Dr. Krimsky asked if the group considered it essential that RAC representatives inspect industrial facilities. Mr. Thornton replied he believed it important that the Director of NIH have the right to designate representatives to visit an industrial site. He recommended that this procedure be continued with other large-scale approvals, not that an inspection would necessarily occur in every case, but that NIH has the authority to do so by consent of the company making the application. Dr. Baltimore agreed with Mr. Thornton's position and asked whether the group felt that the large-scale standards should be flexible. Dr. Walters replied that the revision of the draft standards included a shortening and a simplification.

Dr. Goldstein pointed out that a difference between GMAG and the RAC is that GMAG has labor and worker representatives. Dr. Parkinson said those who had gone on the January 28 visit demonstrated naivete of the industrial world, and that he had never yet come across non-unionized workers who are prepared to make aggressive statements about their workplace conditions. Dr. Baltimore said the January 28 visit was not intended to be a regulatory inspection.

XXI. CONTAINMENT STANDARDS FOR LARGE-SCALE RESEARCH AND PRODUCTION

Dr. Walters noted that tab 862 is a revised draft of the proposed physical containment standards for large-scale work. He then introduced Dr. Emmett Barkley who reported on comments received (tab 835-840, 850, 851, 861) on the earlier (November 14, 1979) draft which had been reviewed at the previous RAC meeting. Dr. Barkley reported that comments on the first draft fell into several broad areas: First, commentators were concerned that procedures for laboratory-scale operations appeared to be extended to large-scale operations. Second, it was suggested that validation procedures be clarified. Third, most commentators placed great emphasis on the containment capability of industrial fermentation vessels. The working group, therefore, attempted to treat the closed fermentation system as the fundamental aspect of containment in large-scale operations and attempted in its November 1979 draft to stipulate only two levels of containment, P2-LS and P3-LS. In response to comments, the working group subsequently concluded that establishing a P1-LS containment level was appropriate. A fourth area addressed by commentators was the monitoring of containment systems. The working group felt it appropriate to establish monitoring requirements on systems that are actually employed. Fifth, the working group also recognized that more attention should be directed to health surveillance. The working group has recommended that health surveillance requirements be a specific recommendation for the P3-LS level.

Dr. Walters suggested that the revised large-scale physical containment standards be published in the Federal Register for an additional period for comment.

Dr. Krimsky said he would present what might be termed a minority report. He said that in his experience with regulation of new technologies at the level of production, the subpopulations at the greatest risk have little or no input into the decision-making process. In his view, it is the moral responsibility of those determining industrial standards to actively seek input from subgroups at potentially greater risk. Yet, his suggestion of bringing representatives of labor before the RAC was denied. He said that the revised standards do not reflect a sufficiently deep analysis of health surveillance. He felt strongly that the Committee should not be involved in what is tantamount to certifying industrial activities, but if the RAC is to be involved, labor should be represented and the RAC should interact directly with the Industrial Practices Subcommittee of the Federal Interagency Committee which has OSHA and NIOSH expertise.

Dr. Young said he was concerned that the RAC is moving more towards a regulatory than an advisory mode. He said he believed the role of the RAC should be to be advisory on scientific principles. He expressed concern that the RAC would become a forum in which industrial procedures are "certified."

Dr. Goldstein said he believed the RAC has exceeded its mandate; in a de facto fashion the RAC is regulating the private sector. He said the RAC does not possess the expertise to evaluate production aspects.

Dr. Williams favored the RAC continuing to play an advisory role in developing large-scale Guidelines, but felt uncomfortable in assessing individual proposals from industry.

Dr. Walters said he believed a need exists for interim standards in an area where no standards currently exist. Dr. Baltimore said he believed the RAC has the responsibility of overseeing the development of recombinant DNA technology. He said the RAC fulfills this function by providing an overview of safety questions. He stressed the time required by OSHA and NIOSH to develop and implement regulations. He congratulated the large-scale working group on proposing an approach relevant to the industrial situation.

Dr. Mason said the taxpayers supporting research expect to obtain some applied benefit from this research. He said the application of recombinant technology to benefit people should not be impeded by a lack of guidelines. He said he agreed that the composition of the RAC is not optimal to evaluate industrial scale-up, but felt that application of the technology should not be delayed because no group is prepared to provide guidelines or regulations. Mr. Thornton said the RAC either possesses, or should be able to obtain, competent advice in industrial application of recombinant technology. Dr. Parkinson said that OSHA possesses the competence and the mandate to deal with this area. Dr. Gottesman suggested that the RAC continue to review submissions for the characterization of clones and to evaluate potential hazards. She said that other procedures could be established for reviewing other aspects of industrial submissions.

Dr. Setlow called on Dr. Christine Oliver of the Oil, Chemical and Atomic Workers International Union to address the RAC. Dr. Oliver said she would reiterate some of the concerns she had expressed in a letter to Dr. Setlow. She said she believed the responsibility for regulating the industrial application of recombinant DNA technology lies with OSHA and NIOSH. The implementation of guidelines for large-scale recombinant DNA technology require a more adequate understanding of the workplace. It is very difficult to visit a plant and obtain any idea of working conditions without an in-depth discussion with the workers themselves. In labor's experience, containment is difficult to attain. As production is scaled up equipment breakdown becomes more frequent. As overtime increases, the number of accidents increase and the subsequent exposure of workers increases. She offered the expertise of her union to the RAC and to OSHA and NIOSH.

Dr. Johnson of Eli Lilly and Company then briefly addressed the RAC. He said that while the RAC may not necessarily have the responsibility of regulating the private sector, the Committee does have the responsibility of overseeing recombinant DNA research regardless of how it is supported. He said he hoped that the RAC will continue an interim approach to industrial projects as jurisdictional authority is sorted out. He commended the working group on the large-scale standards saying they are consistent with good laboratory and good manufacturing practices. He made two suggestions concerning these standards: (1) that "non-debilitated" be defined, and (2) that negative pressure should be required at the P3-LS level rather than at P2-LS.

Mr. Myers of NIOSH said that NIOSH and OSHA are responsible for regulating the workplace. He reported that these two agencies are cooperatively establishing a process in this area. He said these agencies view the RAC as a valuable source of information in the establishment of recommendations. Mr. Pauker of NIOSH noted that all of NIOSH's comments on the earlier draft of the large-scale standards had not been adopted, and that the section on health surveillance is still not to the satisfaction of NIOSH. Mr. Thornton commended the working group and moved that the proposed large-scale standards be published in the Federal Register as recommended Guidelines. Dr. Harris said she supported the motion noting that these standards provide a framework for industry. The motion was passed by a vote of sixteen in favor, two opposed, and one abstention.

Dr. Krimsky then moved that (1) the RAC review only large-scale projects involving no proprietary information at NIH-funded institutions, and (2) the RAC refer other large-scale proposals that do not fall into the above category, with RAC recommendations, to OSHA. Dr. Krimsky said that in this manner the RAC could review the material but would not certify either facilities or proposals. Dr. Baltimore moved to table this proposal. He said he believed the Committee's sentiment was that the RAC should continue to handle these applications and that OSHA was not prepared to handle such proposals at present. The RAC passed the motion to table by a vote of eight in favor, six opposed, and four abstentions.

XXII. PROPOSED LARGE-SCALE EXPERIMENT

Dr. Gottesman introduced the proposal (tab 841) from Dr. Benjamin Hall of the University of Washington. She said that Dr. Hall would like to grow, on a large scale, Saccharomyces cerevisiae carrying a Saccharomyces cerevisiae/Escherichia coli hybrid plasmid containing a Saccharomyces cerevisiae gene. She said this proposal had been considered at a previous meeting but disapproved as the request lacked information on the fermentors. Genentech, Inc., had agreed to grow the cultures for Dr. Hall, but their 75-liter fermentation system had not at that time been evaluated by the RAC.

Dr. Gottesman said she had voted at the previous meeting to approve this proposal as she viewed the experiment as essentially self-cloning. Dr. Walters asked Dr. Gottesman if this experiment could be considered exempt from the Guidelines. Dr. Gottesman responded that the yeast plasmid carries a fragment of *Escherichia coli* DNA and therefore cannot be considered exempt.

Dr. Gottesman recommended that the RAC approve the proposal and so moved. Dr. Young agreed and seconded the motion. Dr. Young said that the yeast sequence is a well-characterized sequence in a well-characterized vector.

The RAC passed this motion by a vote of twelve in favor, none opposed and three abstentions.

XXIII. CLOSED SESSIONS

The RAC went into closed session to consider proposals from commercial concerns for scale-up of recombinant DNA experiments.

XXIV. FUTURE MEETING DATES

The RAC selected the following dates for future meetings:

June 5-6, 1980

September 25-26, 1980

XXV. ADJOURNMENT

The meeting adjourned at approximately 5:15 p.m. Friday, March 7, 1980.

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland Jr.

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

July 8, 1980
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federal register

Monday
April 14, 1980

Part IV

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HEW.

ACTION: Notice of actions under NIH guidelines for research involving recombinant DNA molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724).

EFFECTIVE DATE: April 14, 1980.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496-6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These proposed actions were published for comment in the **Federal Register** of January 31, 1980, and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on March 6-7, 1980. In accordance with Section IV-E-1-b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

Part I of this announcement provides background information on the actions. Part II provides a summary of the major actions.

I. Decisions on Actions Under Guidelines

I-A. Amendment of Section III-O of the Guidelines

In response to a letter of December 21, 1979, from Dr. Stuart Levy of Tufts University the following notice appeared in the **Federal Register** of January 31, 1980:

> "Dr. Stuart Levy of Trufts University School of Medicine has recommended an amendment to Section III-O of the Proposed Revised Guidelines (44 FR 69210). Dr. Levy notes that the plasmid studied in his risk-assessment studies was a nonconjugative poorly mobilizable plasmid. He suggests that a distinction be made between nonconjugative plasmids and nonconjugative plasmids which are also poorly mobilizable; such as amendment would provide additional biological containment. The relevant portion of Section III-O of the Proposed Revised Guidelines which would be amended reads as follows:
>
> * * * Other experiments using *E. coli* K-12 shall use Pl physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system (i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors)* * *. The amended section proposed by Dr. Levy would read as follows (amended text in italic):
>
> * * * Other experiments using *E. coli* K-12 shall use P1 physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system (i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) *lambda or lambdoid bacteriophages or nonconjugative poorly mobilizable (able to be mobilized at a frequency of less than* 10^{8} *by a derepressed conjugative plasmid in the same cell) plasmids shall be used as vectors)** * *."

No comments were received during the thirty day comment period.

This proposed amendment to the language of Section III-O was considered by the RAC at its March 6-7, 1980 meeting. In the discussion it was pointed out that insertion into the Guidelines of the requirement for a "poorly mobilizable" plasmid would then necessitate specification of what tests, under which conditions, must be performed to show that a particular plasmid was or was not "poorly mobilizable." Instead the RAC recommended by a vote of eleven in favor, none opposed and five abstentions, that reference to a new footnote be added to Section III-O, the footnote to read as follows:

> "A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed."

I accept this recommendation.

I-B. Proposed Amendment of Section III-O of the Guidelines

In response to suggestion made in a letter of December 27, 1979, from Dr. Kent Wilcox of the Medical College of Wisconsin, a notice proposing to amend the language of Section III-O was published in the **Federal Register** of January 31, 1980. The relevant part of Section III-O currently reads as follows:

> "* * * An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K-12 efficiently express any gene coding for a eukaryotic protein.* * *"

Dr. Wilcox suggests that for clarity, this sentence be modified to read as follows:

> "* * * An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K-12 efficiently express as a protein product the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.* * *"

During the thirty day comment period, no comments were received.

At the RAC meeting on March 6-7, 1980, it was agreed that the proposed new wording clarifies the intent of this sentence. A motion accepting this amendment to the language of Section III-O was passed by the RAC by a vote of eighteen in favor, none opposed and no abstentions.

I accept this recommendation.

I-C. Request to Include all Species of the Genus Erwinia in Appendix A, Sublist A

In response to a letter of December 27, 1979, the following notice appeared in the **Federal Register** of January 31, 1980:

> "In response to the proposed revised Guidelines published in the **Federal Register** on November 30, 1979 (44 FR 69210), Dr. Clarence Kado of the University of California, Davis, has requested that the NIH consider the inclusion of all *Erwinia* species in Sublist A, Appendix A. Currently only one *Erwinia* species, *E. amylovora*, is included on the list. Dr. Kado suggests that consideration be given to all *Erwinia* species on the basis of an exchange of genetic material by natural means with *E. coli* and a 20% DNA-DNA homology of *E. coli* with *Erwinia* species."

During the thirty day comment period no comments were received.

At the RAC meeting of March 6-7, 1980, the data submitted by Dr. Kado were reviewed. By a vote of fourteen in favor, none opposed and three abstentions the RAC accepted a motion to change the entry in Appendix A, Sublist A of the Guidelines from "*Erwinia amylovora*" to "*Genus Erwinia.*"

I accept this recommendation.

I-D. Request for Approval To Clone Exotoxin A Gene of Pseudomonas Aeruginosa

In a letter of November 19, 1979, Dr. C. W. Shuster of Case Western Reserve University requested that the RAC consider the status under the Guidelines of cloning the exotoxin A gene of *Pseudomonas aeruginosa* in *Escherichia coli*. At its December 6-7, 1979 meeting, the RAC indicated that Dr. Shuster's request appeared to require an exception to a prohibition. Accordingly, the following notice was published in the January 31, 1980 **Federal Register** for a thirty day comment period:

> "Dr. C. W. Shuster of Case Western Reserve University has requested the RAC to consider whether the cloning of the exotoxin

A gene of *Pseudomonas aeruginosa* in *E. coli* K–12 is permitted. Under exemption I–E–4, *P. aeruginosa* and genus *Escherichia* are listed in Appendix A, Sublist A.

However, according to Section I–E, Exemptions:

> It must be emphasized that the following exemptions (4) are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited.

Section I–D–2 prohibits:

> Deliberate formation of recombinant DNA's containing genes for the biosynthesis of toxins potent for vertebrates (2A) (e.g., botulinum, diphtheria toxins; venoms from insects, snakes, etc).
>
> Dr. Shuster notes that exotoxin A and *P. aeruginosa* are safe under normal laboratory conditions. *P. aeruginosa* is an opportunistic pathogen and exotoxin A is only one of a series of virulence factors necessary to produce pathogenicity. This request was presented to the RAC at its December 6–7, 1979 meeting. At that time, the RAC felt that approval of the proposed experiments appeared to require an exception to a prohibition. Accordingly, the request is being published in the **Federal Register** for a thirty day comment period prior to being reconsidered by the RAC at its March 6–7, 1980 meeting."

Prior to the March 6–7, 1980 RAC meeting, two additional requests for permission to perform similar experiements were received.

Dr. Stanley Falkow of the University of Washington requested permission to clone the exotoxin A gene. His long letter of January 18, 1980 included the following arguments: (1) *Pseudomonas aeruginosa* is ubiquitous in nature. (2) *Pseudomonas aeruginosa* does not ordinarily cause disease in healthy individuals. It is a CDC Class 1 agent. (3) Exotoxin A is only one factor in the virulence of *Pseudomonas aeruginosa.* (4) *Pseudomonas aeruginosa* exotoxin A is far less toxic (1 mg. = 5×10^3 mouse LD50 doses) than toxins such as *Clostridium botulinum* a toxic (1 mg. = 1.8×10^8 mouse LD50 doses).

Dr. James Miller of the University of Louisville, Kentucky, in a letter of January 18, 1980, requested permission to clone the *Pseudomonas aeruginosa* exotoxin A gene in *Pseudomonas aeruginosa*.

The RAC at its March 6–7, 1980 meeting once again considered this item. The importance of such experiments for understanding bacterial pathogenicity was stressed. It was agreed that *Pseudomonas aeruginosa* exotoxin A is not as potent a toxin as botulinum or diphtheria toxin. A motion that *Pseudomonas aeruginosa* exotoxin A not be considered a potent toxin, and therefore not be covered under Prohibition I–D–2 passed by a vote of seven in favor, two opposed and ten abstentions. Following the vote it was pointed out that: (a) This would allow total exemption from the Guidelines under Sections I–E–3 and I–E–4 respectively for the cloning of the *Pseudomonas aeruginosa* exotoxin A gene in *Pseudomonas aeruginosa* and *Escherchia coli;* and (b) this was more than any of the investigators were actually requesting. A motion to reconsider the previous vote passed by a vote of fifteen in favor, non opposed and two abstentions. A motion to permit the cloning of *Pseudomonas aeruginosa* exotoxin A in *E. coli* K–12 at Pl + EK1 containment passed by a vote of fourteen in favor, non oppossed, and three abstentions.

I–E. Classification of Plant Pathogens

The following notice appeared in the **Federal Register** of January 31, 1980:

> "In response to the proposed revised Guidelines for Research Involving Recombinant DNA Molecules published in the **Federal Register** on November 30, 1979 (44 FR 69210), M.T. Goff, Animal and Plant Health Inspection Service, United States Department of Agriculture, has proposed that a classification of plant pathogens be included in the Guidelines. Philip D. Harriman, National Science Foundation, has also submitted a similar proposal. Messrs. Goff and Harriman request that a portion of the Report of a Workshop on Risk Assessment of Agricultural Pathogens, dealing with a hazard classification of plant pathogens, be included in the Guidelines. This Workshop was held on March 20–21, 1978, and the report appeared as Appendix G to the Environmental Impact Assessment which accompanied proposed revised Guidelines published for comment in the **Federal Register** of July 28, 1978 (43 FR 33042). Specifically, they request that the following classification be added to Appendix B of the Guidelines.
>
> IV. Plant Pathogens.
>
> Class 1A Agents—Plant pathogens not in Class 1B.
>
> Class 1A Agents—All organisms that are subject to quarantine restrictions for any of the following reasons:
>
> (i) Plant pathogens not known to occur in the United States.
>
> (ii) Plant pathogens that are not widely distributed throughout the ecological range of their hosts in the United States.
>
> (iii) Plant pathogens subject to U.S. Federal or State eradication or suppression programs.
>
> A USDA permit [3] is required to import or to move plant pathogens across State lines.
>
> Footnotes 2 of Appendix B of the proposed revised Guidelines reads as follows:
>
> > USDA permit also required for import and interstate transport.
>
> It is proposed that this footnote be modified to read as follows in order to serve as a single footnote for both animal and plant pathogens:
>
> [3] A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

During the thirty day comment period, no comments were received.

The RAC discussed this item at its March 6–7, 1980 meeting, and determined that the addition of the proposed classification of plant pathogens to Appendix B of the Guidelines was unnecessary. Rather, the RAC suggested by a vote of twenty in favor, none opposed, and no abstentions, that the footnote

> A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782."

be added to Section V of the Guidelines and cited wherever the Guidelines refer to plant pathogens.

I accept this recommendation.

I–F. Request for Consideration of Appropriate Containment Conditions

The following notice was published in the **Federal Register** of January 31, 1980:

> "Dr. Olen Yoder of Cornell University has asked the RAC to consider the appropriate containment level for the return of *Helminthosporanium maydis* DNA, which has been cloned in *Saccharomyces cerevisiae,* to the host of origin."

During the thirty day comment period, no comments were received.

The RAC at its March 6–7, 1980 meeting discussed this proposal. *Helminthosporanium maydis* is a fungus which grows on corn. Work will be done with the strain called race O. The vector will be the hybrid *E. coli*-yeast plasmid Ylp5. This is a "return to host of origin" type experiment with the intermediate host being yeast strain SHY2. The RAC by a vote of eight in favor, none opposed and nine abstentions accepted a P2 level of containment for the proposed experiments.

I accept this recommedation.

I–G. Request for Consideration of Appropriate Containment Conditions

In response to a request of December 13, 1979 to set containment conditions for experiments involving *Schizophyllum commune,* the following notice was placed in the **Federal Register** of January 31, 1980:

> "Dr. Marvin Schwalb of New Jersey Medical School has requested that the RAC consider the containment levels appropriate to the return of *Schizophyllum commune* DNA cloned in *Saccharomyces cerevisiae* to *Schizophyllum commune.* In addition, Dr. Schwalb requests permission to clone the *S. cerevisiae* derived vector YR414/ura 3 and

> the *Saccharomyces* 2 mu plasmid containing yeast or *S. commune* sequences in *S. commune.*"

During the thrity day comment period, no comments were received.

The RAC at its March 6–7, 1980 meeting discussed this item. It was noted that *Schizophyllum commune* is a nonpathogenic saprophytic basidomycete which occurs widely in nature. The proposed experiments were judged to pose no hazard. The RAC by a vote of eighteen in favor, none opposed and one abstention, accepted a P2 level of containment for the proposed experiments.

I accept this recommendation.

I–H. Request for Consideration of Appropriate Containment Conditions

In response to a request of December 7, 1979, to clone *Wangiella dermatitidis* DNA, the following notice was published in the **Federal Register** of January 31, 1980:

> "Dr. Charles Jacobs of the University of Texas at Austin has requested permission from the RAC to clone *Wangiella dermatitidis* DNA in *Wangiella dermatitidis* using *Saccharomyces/E. coli* hybrid plasmids as vectors. Dr. Jacobs requests that the RAC assess appropriate containment conditions."

During the thirty day comment period, no comments were received.

The RAC discussed this item during its March 6–7, 1980 meeting. *Wangiella dermatitidis* is a fungus which can in rare instances cause a deep mycosis in humans. It is classified by the Center for Disease Control as a Class 2 agent. This is essentially a "return to host of origin" type experiment. With the proviso that a HV2 certified *Saccharomyces/E. coli* hybrid plasmid vector be employed in the experiments, the RAC by a vote of ten in favor, none opposed and four abstentions recommended that the experiments be approved at the P3 level of containment.

I accept this recommendation.

I–I. Proposed Exemption for Streptococcus Mutans and Streptococcus Sanguis

The following notice was published in the **Federal Register** of January 31, 1980:

> "Dr. Francis Macrina of the Virginia Commonwealth University has proposed that *Streptococcus mutans* and *Streptococcus sanguis* be included under the exemption category of Section I–E–4 of the Guidelines on the basis that these bacteria have been shown to exchange chromosomal DNA by known physiological processes. These two organisms would then constitute a new Sublist of Appendix A."

No comments were received during the thirty day comment period.

At the March 6–7, 1980 meeting, the RAC discussed the data submitted by the investigator in support of his request. It was agreed that the documentation is good showing natural genetic exchange of chromosomal genes from *Streptococcus mutans* to *Streptococcus sanguis*. It was pointed out that the Director, NIH, had previously endorsed the concept of "one-way lists" for Appendix A (**Federal Register**, December 22, 1976, Page 60100). The RAC recommended by a vote of nineteen in favor, none opposed, and no abstentions, that a new sublist be added to Appendix A of the Guidelines exempting recombinant DNA experiments involving the one-way transfer of *Streptococcus mutans* DNA into *Streptococcus sanguis*.

I accept this recommendation.

I–J. Proposed Revision of Section III–C–1–e and Its Subsections

The following notice appeared in the **Federal Register** of January 31, 1980:

> "The proposed revised Guidelines (44 FR 69210), in Appendix C, cite a limited exemption, under Section I–E–5, for certain recombinant DNA experiments in tissue culture:
>
> Under exemption I–E–5 of these Revised Guidelines are those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus).

This exemption was promulgated in the **Federal Register** of July 20, 1979 (44 FR 42914). In its original formulation (44 FR 22314), the exemption proposed to include recombinant DNA molecules that contain no more than one-fourth of the genome of a eukaryotic virus. At its December 6–7, 1979 meeting, Dr. Wallace Rowe submitted a document requesting that the RAC reconsider the question of allowing exemption for tissue culture experiments involving recombinant molecules containing no more than one-fourth of the genome of a virus. A working group was appointed to make recommendations for consideration at the March 6–7, 1980 meeting. It has been recommmended that Sections III–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e–(1)–(b) of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The proposed revised sections would read as follows:

> III–C–1–e. All Viral Vectors.
>
> III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:
>
> III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture in the absence of helper vurus using P1 containment. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome. For such experiemnts, no MUA need be submitted but prior notice must be given to the IBC as described in Section III–O of the Guidelines. The IBC should handle such registration documents as described in Section III–O.
>
> III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.
>
> III–C–1–e–(1)–(c). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild type strains of the helper virus are not able to grow in human cells.

During the thirty day comment period no comments were received.

At the March 6–7, 1980 meeting, the RAC discussed this proposal. In support of the proposal it was stated that there is no experimental evidence to date that any eukaryotic virus can dispense with one-third of its genetic information and replicate autonomously or that less than two-thirds of viruses from different Families can fully complement each other's genetic composition.

During the discussion it was recommened that the proposed Section III–C–1–e–(1)–(a) as published in the **Federal Register** on January 31, 1980 should be further modified in two ways:

The last two sentences of proposed Section III–C–1–e–(1)–(a) should be deleted. (Deletion means a more conservative review procedure for these experiments).

In the first sentence, the words "in the absence of helper virus" should be deleted. A new second sentence should be added as follows: "It must be shown that the cells lack helper virus for the specific Families of the defective viruses being used." (This change means a more conservative standard concerining the presence of helper viruses.)

A motion that RAC accept Sections III–C–1–e, III–C–1–e–(1), as III–C–1–e–(1)–(a) as published in the **Federal Register** on January 31, 1980, with the two modifications in Section III–C–1–e–(1)–(a) as noted above, passed by a vote of thirteen in favor, three opposed, and five abstentions. A vote to defer consideration until the next RAC meeting of the proposed new Sections III–C–1–e–(1)–(b) and III–C–1–e–(1)–(c) as published in the **Federal Register** on January 31, 1980 passed by a vote of thirteen in favor, five opposed and one abstention.

During the discussion it was also suggested that in Section III–C–1–e–(1)–(a), after the word "Family," reference to footnote 36 be added, and that footnote 36 be revised to make reference to the third report of the International Committee on Taxonomy of Viruses.

I accept the recommendations of the RAC, i.e., Footnote 36 will be revised and new Sections III–C–1–e, III–C–1–e–(1), and III–C–1–e–(1)–(a) as recommended by the RAC at their March 6–7, 1980 meeting will replace the previous Sections so numbered. Sections III–C–1–e–(1)–(b) and III–C–1–e–(2) remain identical in wording (**Federal Register**, January 29, 1980), but they now fall under a new heading III–C–1–e. "All Viral Vectors," rather than as previously III–C–1–e. "All Other Potential Viral Vectors."

In addition for clarification, I am adding reference to a new footnote 50 in Section III–C–1–e–(1)–(a) after the words "* * * considered identical)." Footnote 50 will read "i.e. the total of all genomes within a Family shall not exceed two-thirds of the genome."

II. Summary of Major Actions Under Guidelines

II–A. Amendment of Section III–O of the Guidelines and New Footnote 49

A new footnote 49 is added to section V of the Guidelines, as follows:

> "[49] A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed."

The fourth paragraph of Section III–O of the Guidelines is modified to add reference to Footnote 49, as follows:

> "Other experiments using E. coli K–12 shall use P1 physical containment and, except as specified in the last paragraph of this section, an EK1 host-vector system [i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids (49) shall be used as vectors]. For these experiments * * *"

II–B. Amendment of Section III–O of the Guidelines

Section III–O (fourth paragraph, last sentence) of the Guidelines is amended to change the words "* * * have the *E. coli* K–12 efficiently express any gene coding for a eukaryotic protein." to "* * * have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived either from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism."

II–C. Inclusion of Genus Erwinia in Appendix A, Sublist A

The entry "*Erwinia amylovora*" in Appendix A, Sublist A of the Guidelines is changed to "Genus *Erwinia*."

II–D. Approval to Clone Exotoxin Gene of Pseudomonas Aeruginosa

Added to Appendix E of the Guidelines is the following: "Permission is granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under P1 + EK1 conditions in *Escherchia coli* K–12 and under P1 conditions in *Pseudomonas aeruginosa*."

II–E. New Footnote 48 Concerning Plant Pathogens

A new footnote 46 is added to Section V of the Guidelines, as follows:

> "[46] A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782."

Reference to footnote 46 is added as given below to the following sections of the Guidelines:

> "I–D–3. Deliberate creation by the use of recombinant DNA of a plant pathogen (46) with increased virulence and host range beyond that which occurs by natural genetic exchange (2A)."
>
> "III–A–2–a–(1)–(a)–(*l*). Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (Strain FL) and Plant Viruses (46) * * *"
>
> "III–A–2–a–(2)–(e). RNA Plant Viruses and Plant Viroids (46) * * *"
>
> "III–C–4. Plant Host-Vector Systems Other than Viruses (46) * * *"

In addition the footnote given at the bottom of the first column of pg. 6748 of the Guidelines (**Federal Register**, January 29, 1980) is changed to:

> "A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD. 20782."

II–F. Return of Helminthosporanium Maydis DNA to Helminthosporanium Maydis

Added to Appendix E of the Guidelines is the following:

> "Permission is granted to return to the host of origin *Helminthosporanium maydis* (race O) DNA which has been cloned in yeast strain SHY2 using the hybird *E. coli*—yeast plasmid Y1p5. The cloned DNA may be returned, to and propagated in, *Helminthosporanium maydis* at the P2 level of physical containment."

II–G. Return of Schizophyllum Commune and Saccharomyces Cerevisiae DNA to Schizophyllum Commune

Added to Appendix E of the Guidelines is the following:

> "Permission is granted to return *Schizophyllum commune* DNA (or yeast DNA) cloned in *Saccharomyces cerevisiae* with YR or 2 mu circle vectors to *Schizophyllum commune*. The cloned DNA may be retunred to, and propagated in, *Schizophyllum commune* at the P2 level of physical containment."

II–H Return of Wangiella Dermatitidis DNA to Wangiella Dermatitidis

Added to Appendix E of the Guidelines is the following:

> "Permission is granted to return *Wangiell dermatitidis* DNA to *Wangiella dermatitidis* using an HV2 certified *Sacchromyces/E. coli* hybrid vector. The *Wangiella dermatitidis* may be propagated at the P3 level of physical containment."

II–I. Streptococcus Mutans and Streptococcus Sunguis as a New Sublist in Appendix A

A new sublist is added to Appendix A of the Guidelines as follows:

> "Sublist E—One way transfer of *Streptococcus mutans* DNA into *Streptococcus sanguis*."

II–J. Revision of Guidelines Section III–C–1–e and Its Subsections

Revision of Footnote 36. Addition of New Footnote 50. A new footnote number 50 is added to Section V of the Guidelines as follows:

> "[50] i.e. the total of all genomes within a Family shall not exceed two-thirds of the genome."

Footnote 36 of Section V of the Guidelines is revised by changing the first sentence to:

> "As classified in the Third Report of International Committee on Taxonomy of viruses Classification and Nomenclature of Viruses, R. E. F. Matthews, Ed. Intervirology 12 (129–296) 1979."

Section III–C–1–e, III–C–1–e–(1) and III–C–1–e–(1)–(a) of the Guidelines are revised as follows:

> "III–C–1–e, All Viral Vectors.
>
> III–C–1–e–(1). Other experiments involvi eukaryotic virus vectors can be done as follows:
>
> III–C–1–e–(1)(a). Recombinant DNA molecules containing no more than two-thi of the genome of any eukaryotic virus [all viruses from a single Family (36) being considered identical (50)] may be propaga and maintained in cells in tissue culture u P1 containment. For such experiments, it must be shown that the cells lack helper vi for the specific Families of defective viruse being used. The DNA may contain fragme of the genomes of viruses from more than

Family but each fragment must be less than two-thirds of a genome."

Additional Change in the Guidelines

In addition to the changes in the Guidelines recommended at the March 6–7 RAC meeting, one further change is hereby promulgated to correct a printing error. Section IV–D–1–c was printed incorrectly in the **Federal Register** of January 29, 1980 (45 FR 6739) where it therefore incorrectly appears that the "Note" within the Section consists of two sentences. Actually the Note should consist of only one sentence, the succeeding sentence not being part of the Note. Section IV–D–1–c had been printed correctly in the **Federal Register** of November 30, 1979 (44 FR 69225). The correct version of Section IV–D–1–c is as follows:

"IV–D–1–c. Submit, for each recombinant DNA project that meets with its approval, a Memorandum of Understanding and Agreement (MUA) to the funding agency for approval and registration. (Note: No MUA is required for experiments described in Section III–O). All projects, however, can proceed upon IBC approcal (before submission of the MUA to the funding agency) except for the following, which require prior approval by NIH (or other funding agency designated by NIH for this purpose):"

Dated: April 8, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

[FR Doc. 80-11097 Filed 4-11-80; 8:45 am]

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National Institutes of Health

National Institute of Allergy and Infectious Diseases; Meeting

Notice is hereby given of a Workshop to consider two areas within the NIH Program to Assess the Risks of Recombinant DNA Research. This Workshop is being sponsored by the National Institute of Allergy and Infectious Diseases as part of its responsibility to implement the Program. The meeting will be convened at the Huntington Sheraton Hotel, Pasadena, California on April 11 and 12, 1980.

This Workshop is designed to define the scientific issues and assess the potential risks of (1) possible direct adverse effects of hormone-producing strains of *E. coli* K-12, and (2) the possible occurrence of autoantibodies or autoreactive cells due to the production of eukaryotic polypeptides (including hormones) by *E. coli* K-12 should they colonize higher organisms. These potential risks are being considered because there is still debate over the degree of possible risk, even though *E. coli* K-12 has apparently lost those known characteristics that are required for colonization of the normal intestinal tract. The meeting will bring together outstanding scientists from the fields of immunology, endocrinology, physiology, microbiology, infectious diseases and other appropriate disciplines. The entire meeting is open to the public but such attendence is limited to the space available.

Further detailed information can be obtained from Dr. John E. Nutter, Chief, Office of Specialized Research and Facilities, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, (301) 496-5643.

Dated: February 15, 1980.

Suzanne L. Fremeau,

Committee Management Officer, National Institutes of Health.

[FR Doc. 80-[illegible] Filed 2-25-80; 8:45 am]

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federal register

Friday
April 11, 1980

Part II

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Research; Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules

AGENCY: National Institutes of Health (NIH), HEW.

ACTION: Publication of physical containment recommendations for large-scale uses of organisms containing recombinant DNA molecules.

SUMMARY: These physical containment recommendations for large-scale uses of organisms containing recombinant DNA molecules are published for public information in accordance with the recommendation of the NIH Recombinant DNA Advisory Committee.

FOR FURTHER INFORMATION CONTACT: Dr. W. Emmett Barkely, Director, Division of Safety, Building 13, Room 2E45, National Institutes of Health, Bethesda, Maryland 20205.

SUPPLEMENTAL INFORMATION: The NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724, January 29, 1980) specify in Section IV–E–1–b–(3)–(d) that the Director, NIH, is responsible for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

At its September 6–7, 1979, meeting, the NIH Recombinant DNA Advisory Committee (RAC) adopted (by a vote of 14 in favor, none opposed, and 3 abstentions) procedures to be followed in reviewing such large-scale experiments. These procedures included the submission by the applicant of certain information including "a description of the applicant's laboratory practices, containment equipment, and facilities rlevant to the containment of large volumes of culture."

At its May 21–23, 1979, meeting, the RAC approved (by a vote of 19 in favor, none opposed, and no abstentions) the creation of a working group to "examine physical containment requirements for large-scale experiments and that a document should be prepared."

At its December 6–7, 1979, meeting, the RAC reviewed Draft Physical Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules and voted (15 in favor, none opposed, and no abstentions) that additional comments be solicited on the draft standards.

At its March 6–7, 1980, meeting, the RAC reviewed Draft Physical Containment Guidelines for Large-Scale Use of Organisms Containing Recombinant DNA Molecules which had been revised from the earlier draft, based upon comments received. Following considerable discussion at the meeting, a motion was passed (with 16 in favor, 2 opposed, and 1 abstention) that the document be published in the **Federal Register**.

The document which is now published in the **Federal Register** contains changes from that reviewed by the RAC on March 7, 1980, based upon suggestions made at the March 7 meeting. These changes are:—(1) To eliminate what was Section VII–C–6 in the draft reviewed by the RAC on March 7, 1980; Section VII–D–6 which consists of identical text has been retained.—(2) To change the phrase "using a non-debilitated strain of the host organisms used in the research or production process" to "using the organism that will serve as the host for propagating the recombinant DNA molecules" in Section VII–B–2, VII–B–5, VII–C–2, VII–C–5, VII–C–8, VII–D–2, VII–D–5, and VII–D–9.

When earlier drafts of this document had been prepared it was proposed that it might eventually be formally promulgated as Part VII of the NIH Guidelines for Research Involving Recombinant DNA Molecules. It has not in fact been formally adopted as Part VII of the NIH Guidelines. The format has, however, been retained in this *Draft* Part VII. This document, while not formally part of the NIH Guidelines, can serve as a model for those preparing submissions to the NIH under the NIH Guidelines.

The document now published in the **Federal Register** is endorsed by the NIH Division of Safety as physical containment recommendations appropriate for large-scale uses of organisms containing recombinant DNA molecules.

Dated: March 31, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

Draft Part VII of the NIH Guidelines for Research Involving Recombinant DNA Molecules

VII. Physical Containment for Large-Scale Uses of Viable Organisms Containing Recombinant DNA Molecules

This part of the NIH Guidelines specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to all large-scale research or production activities approved by the Director, NIH in accordance with Sections IV–E–2–b–(1)–(e) and IV–E–1–b–(3)–(d) of the NIH Guidelines.

All provisions of the NIH Guidelines shall apply to large-scale research or production activities with the following modifications:

- Part VII shall replace Section II–B when quantities in excess of 10 liters of culture are involved in research or production.
- The institution shall appoint a biological safety officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the biological safety officer shall include those specified in Section IV–D–4.
- The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require P3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual or extended illnesses of employees to determine possible occupational origin.

VII–A. *Selection of Physical Containment Levels.* The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Part III of the Guidelines. For purposes of large-scale research or production, three physical containment levels are established. These are referred to as P1–LS, P2–LS, and P3–LS. The P1–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require P1 containment at the laboratory scale. The P2–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require P2 containment at the laboratory scale. The P3–LS level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules which require P3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant DNA molecules which require P4 containment at the laboratory scale. If necessary, these requirements will be established on an individual case basis.

VII–B. *P1–LS Level.*

VII–V–1. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment providing all physical containment requirements specified in Section II–B–1. of the Guidelines are met.

VII–B–2. Culture fluids (except as allowed in VII–B3.) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

VII–B–3. Sample collection from a closed system, the addition of materials to a closed system and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

VII–B–4. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

VII–B–5. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

VII–B–6. Emergency plans required by Section IV–D–3–d. shall include methods and procedures for handling large losses of culture on an emergency basis.

VII–C. *P2–LS Level.*

VII–C–1. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment providing all physical containment requirements specified in Section II–B–2. of the Guidelines are met.

VII–C–2. Culture fluids (except as allowed in VII–C–3.) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

VII–C–3. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

VII–C–4. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g. incinereation) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

VII–C–5. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opended for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

VII–C–6. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhasted through filters which have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

VII–C–7. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

VII–C–8. A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecues, and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

VII–C–9. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to the use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

VII–C–10. The universal biohazard sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

VII–C–11. Emergency plans required by Section IV–D–3–d shall include methods and procedures for handling large losses of culture on an emergency basis.

VII–D. *P3–LS Level.*

VII–D–1. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system providing all physical containment requirements specified in Section II–B–3 of the Guidelines are met.

VII–D–2. Culture fluids (except as allowed in VII–D–3) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

VII–D–3. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be done in a manner which prevents the release of aerosols or contamination of exposed surfaces.

VII–D–4. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g. incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

VII–D–5. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

VII–D–6. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at or slightly below atmospheric pressure.

VII–D–7. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to HEPA filters or through other equivalent treatment devices.

VII–D–8. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

VII–D–9. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules, and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

VII–D–10. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation and maintenance and in all documentation relating to the use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

VII–D–11. The universal biohazard sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

VII–D–12. Emergency plans required by Section IV–D–3–d shall include methods and procedures for handling large losses of culture on an emergency basis.

VII–D–13. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

VII–D–13–a. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom or change room that separates the controlled area from the balance of the facility.

VII–D–13–b. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

VII–D–13–c. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

VII–D–13–d. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

VII–D–13–e. Handwashing facilities equipped with foot-, elbow-, or automatically-operated valves shall be located at each major work area and near each primary exit.

VII–D–13–f. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

VII–D–13–g. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

VII–D–13–h. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

VII–D–14. The following personnel and operational practices shall be required:

VII–D–14–a. Personnel entry into the controlled area shall be through the entry area specified in section VII–D–13–a.

VII–D–14–b. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jumpsuits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

VII–D–14–c. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

VII–D–14–d. Persons under 18 years of age shall not be permitted to enter the controlled area.

VII–D–14–e. The universal biohazard sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

VII–D–14–f. The controlled area shall be kept neat and clean.

VII–D–14–g. Eating, drinking, smoking and storage of food are prohibited in the controlled area.

VII–D–14–h. Animals and plants shall be excluded from the controlled area.

VII–D–14–i. An effective insect and rodent control program shall be maintained.

VII–D–14–j. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Service doors leading directly outdoors shall be sealed and locked while work is in progress.

VII–D–14–k. Persons shall wash their hands when leaving the controlled area.

VII–D–14–l. Persons working in the controlled area shall be trained in emergency procedures.

VII–D–14–m. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

VII–D–14–n. The Controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

[FR Doc. 80–10280 Filed 4–10–80; 8:45 am]
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federal register

Wednesday
April 30, 1980

Part II

Department of Health, Education, and Welfare

National Institutes of Health

Recombinant DNA Research; Announcement of Meeting of Advisory Committee and Notice of Proposed Actions Under Guidelines

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92-463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Conference Room 6, Building 31C, Bethesda, Maryland 20205, on June 5, 1980, from 9:00 a.m. to recess at approximately 6:00 p.m., and on June 6, 1980, from 8:30 a.m. to 5:00 p.m. This meeting will be open to the public on June 5 from 9:00 a.m. to recess at approximately 6:00 p.m., and on June 6 from 8:30 a.m. to approximately 3:00 p.m. to discuss:

Amendment of Guidelines
Containment practices appropriate for large-scale production
Voluntary compliance with Guidelines
Institutional Biosafety Committees
Exceptions to prohibitions
Exemptions for organisms that exchange genetic information
Procedures for lowering containment for characterized clones and purified DNA
Review of large-scale proposals
E. coli K-12 host-vector systems
Host-vector systems other than *E. coli* K-12
NIH risk-assessment plan
Review of protocols for required containment levels
Other matters requiring necessary action by the Committee.

Attendance by the public will be limited to space available.

In accordance with provisions set forth in section 552b(c)(4), Title 5, U.S. Code and section 10(d) of Pub. L. 92-463, the meeting will be closed to the public for approximately two hours for the review, discussion and evaluation of proposal(s) from a commercial concern(s) for scale-up of recombinant DNA experiments. It is anticipated that this will occur on June 6, from approximately 3:00 p.m. until adjournment. The proposal(s) and the discussion could reveal confidential trade secrets or commercial property such as patentable material.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 4A52, telephone 301-496-6051, will provide materials to be discussed at the meeting, rosters of committee members and substantive program information. A summary of the meeting will be available at a later date.

Dated: April 22, 1980.

Suzanne L. Fremeau,

Committee Management Officer, National Institutes of Health.

[FR Doc. 80-13046 Filed 4-29-80; 8:45 am]

BILLING CODE 4110-08-M

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHEW.

ACTION: Notice of proposed actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposals for actions to be taken under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules [Federal Register of January 29, 1980 (45 FR 6724)]. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its June 5-6, 1980, meeting, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by May 30, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Director, office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496-6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724), as well as actions under these Guidelines.

1. *Proposal to Amend Section III-O of the Guidelines to Permit Use of Ff Phages.* The National Institutes of Health (NIH) promulgated revised Guidelines in January 1980 (45 FR 6724). The revised Guidelines include a new Section, III-O, "Classification of Experiments Using the *E. coli* K-12 Host-Vector Systems," which, in part, reads as follows.

> "* * * experiments using *E. coli* K-12 shall use P1 physical containment and * * * an EK1 host-vector system [i.e. (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors].* * *"

Section III-O, which was promulgated on the recommendation of the RAC, did not include reference to use of Ff bacteriophages (filamentous single-strand male-specific bacteriophages such as M13 and fd). Consequently, when the revised Guidelines were promulgated in January 1980, investigators were informed that Ff bacteriophages may continue to be used as a component of EK1 systems at the containment levels specified by the 1978 Guidelines. (A complete discussion of this issue appears in the Decision Document that accompanied the November 30, 1979 proposed revised Guidelines (44 FR 69244-69245) and the Decision Document that accompanied the final revised Guidelines (45 FR 6721)).

In December 1979, the RAC was asked to consider how *E. coli* K-12 host-vector systems employing Ff phages should be treated under the 1980 Guidelines. In response to this NIH request, the Host-Phage Subcommittee drafted a recommendation which was presented to the full RAC at the March 6-7, 1980 meeting.

The RAC agreed that the following proposed amendment to Section III-O should be published in the Federal Register for a thirty-day comment period (amended text in italics):

> "* * * experiments using *E. coli* K-12 shall use P1 physical containment and, * * * an EK1 host-vector system [i.e. (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid *or Ff* bacteriophages or non-conjugative plasmids shall be used as vectors].* * *"

Background documentation is available from ORDA.

2. *Proposed Changes to Allow Recombinant DNA Experiments with Class 3 Organisms and Certain Plant Pathogens.* The National Institute of Allergy and Infectious Diseases (NIAID) requested that the NIH consider whether, and under what conditions, recombinant DNA technology might be used to study CDC Class 3 etiological agents. Experiments employing these agents are currently prohibited by Section I-D-1 of the Guidelines which states as not to be initiated:

> "I-D-1. Formation of recombinant DNMAs derived from the pathogenic organisms classified [1] as Class 3, 4 or 5 [2] or the cells

known [2A] to be infected with such agents, regardless of the host-vector system used."

This question was presented to the RAC at its December 6–7, 1979 meeting and a working group was appointed to study the issue. A draft proposal of a revision of the Guidelines to remove Class 3 agents from Section I–D–1 of the Guidelines was presented to the RAC at its March 6–7, 1980 meeting.

Prior to the March 6–7, 1980 meeting, Dr. Clarence Kado of the University of California, Davis, requested the RAC to consider the elimination of Section I–D–3 of the Guidelines, which states as not to be initiated:

"I–D–3. Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange. [2A]"

This issue was also discussed by the RAC at the March 6–7, 1980 meeting. During the discussion, it was suggested that the mechanism used to remove CDC Class 3 agents from Section I–D–1 of the Guidelines might be appropriate for removing recombinant DNA experiments involving certain plant pathogens from Section I–D–3. Accordingly the proposals have been combined.

The purpose of the proposed changes is to remove recombinant DNA experiments with CDC Class 3 organisms and certain recombinant DNA experiments with plant pathogens from the prohibited classes of experiments. The proposal would allow experiments with CDC Class 3 organisms to be conducted at P3 containment in *E. coli* K–12 EK1 host-vector systems. The containment levels for other recombinant DNA experiments would be assigned by the director, NIH after review by the RAC.

It is felt that reducing administrative procedures will increase flexibility and encourage the use of these techniques in elucidating the pathogenic process. P3 containment, which is considered adequate for work with the pathogenic organisms themselves, should be adequate, or more than adequate, for work with *E. coli* K–12 containing recombinant DNA from these organisms.

Changes are proposed in the Guidelines as follows:

A. Section I–D–1 is to be amended to read:

"I–D–1. formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known [2A] to be infected with such agents, regardless of the host-vector system used."

B. Section I–D–3 is to be deleted.

C. Section I–E is to be amended to read:

"I–E. *Exemptions.* It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5."

D. Section III. After the last sentence, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:

"Experiments involving recombinant DNA from Class 3 organisms (1) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen will be determined by NIH (See Section IV–E–1–b–2–e)."

E. Section III–O. After the third paragraph, "Some experiments * * * are exempt from the Guidelines (see Section I–E)," the following new paragraph is to be added:

"Experiments using *E. coli* K–12 EK1 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–3–1–b–2–e)."

F. A new Section IV–E–1–b–2–e is to be added as follows:

"IV–E–1–b–2–e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and for experiments which increase the host-range and virulence of plant pathogens."

G. Section V, footnote 2, is to be amended to read:

"2. For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of recombinant DNA Activities."

H. Section V, footnote 36. The second sentence is to be amended to read:

"(As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)"

3. *Proposal to Amend Portions of Section III–A–2–a, Viruses of Eukaryotes.* In the 1980 Guidelines a level of biological containment requiring the use of an "HV1 host and a vector certified for use in an HV2 system" has been substituted for "an EK1 host and a vector certified for use in an EK2 system." This level of biological containment is abbreviated as "HV1CV" in Table III and Footnote 40 of the 1980 Guidelines. At the December 6–7, 1979 meeting of the RAC, a working group was appointed to review the appropriateness of this level of biological containment. The working group proposed that HV2 biological containment be required for these experiments. Accordingly, a proposal to amend portions of Section III–A–2–a, *Viruses of Eukaryotes,* was published in the **Federal Register** of January 31, 1980 [45 FR 7182].

The RAC discussed this proposal at the March 6–7, 1980 meeting. During the discussion, the RAC agreed that the "CV" nomenclature was developed for EK host-vector systems. However the RAC felt that requiring the use of HV2 systems instead of HV1CV imposed an unnecessarily stringent level of containment.

The RAC voted to publish for comment in the **Federal Register** a proposal to substitute HV1 for HV1CV in all places in the Guidelines. The proposal is as follows: Substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(*3*)–(*b*) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(*2*)–(*c*), III–A–2–a–(1)–(b)–(*1*)–(*b*), III–A–2–a–(1)–(b)–(*2*)–(*b*), III–A–2–a–(2)–(a)–(*1*)–(*b*), III–A–2–a–(2)–(a)–(*2*)–(*b*), and III–A–2–a–(2)–(c)–(*2*)–(*b*). In Table III, whenever any one of the following three terms appears, ("HV1CV [40]"; HV1CV[40] or P3 + HV1"; or "HV1CV[40] or P3 + HV1[38]") it would be changed to "HV1."

4. *Proposal To Include Saccharomyces cerevisiae Host-Vector Systems under Section III–O of the Guidelines.* Dr. Jane Setlow of the Brookhaven National Laboratory, proposed at the March 6–7, 1980 meeting, that the RAC consider classifying under Section III–O of the Guidelines experiments involving laboratory strains of *Saccharomyces cerevisiae* as host-vector systems. Dr. Setlow advanced the following arguments to support this proposal: (1) *S. cerevisiae* is nonpathogenic; (2) it does not implant in the intestine; (3) the dilute conditions in which it is found in nature are extremely unfavorable for mating; and (4) it does not efficiently compete with wild type strains of *S. cerevisiae.* She noted in addition that *S. cerevisiae* does not have a biological association with man. Material supporting this proposal has been provided to ORDA by Dr. Setlow and is available upon request. The RAC discussed this question at the March 6–

7, 1980 meeting, and agreed that the proposal should be published in the **Federal Register** for comment.

The proposal consists of two parts as follows:

A. The following language would be substituted for Section III–O:

> III–O *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems.
>
> These are the systems for which we have the most experience and knowledge.
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).
>
> Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, and HV1 host-vector system [i.e. for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids [40] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used.] For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their intiation, investigators must submit to their Instutitional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to intiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 or *S. cerevisiae* efficiently express as a protein product the information carried in any gene derive from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.
>
> Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).
>
> For those not on the Appendix A list but which exchange genetic information (35) with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g. conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

B. The language "*HV1—Unmodified laboratory strains of *Saccharomyces cerevisiae*" would be deleted from Appendix D.

5. *Request for Permission to Transform Sacchromycopsis lipolytica with E. coli/S. cerevisiae Hybrid Plasmids.* Dr. David M. Ogrydziak of the University of California, Davis, requests permission to attemt to transform the yeast *Sacchromycopsis lipolytica* with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and to use the hybrid plasmids for shotgunning *S. lipolytica* DNA in *E. coli* and returning the DNA to *S. lipolytica.*

6. *Proposal to Amend Section III–B–3 of the Guidelines.* Dr. Allan Campbell of Stanford University has proposed that the RAC consider amending Section III–B–3 of the Guidelines, which currently reads as follows:

> III–B–3. Non-HV1 systems. Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).
>
> In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment. (2A)

Dr. Campbell writes that the P3 containment level was assigned because of apprehensions that the category might contain some experiments for which that level was appropriate rather than that all experiments in this category were perceived as requiring P3 containment. His proposed amendments are "intended to assert RAC's intention to review individual proposals in an open-minded manner with respect to realistic evaluation of possible hazard, and to discourage the inference that the P3 level, as it applies to a specific experiment, has been set on the basis of information about the organisms involved."

Dr Campbell proposes changes in the Guidelines as follows:

A. Section III–B–3, paragraph 2, would be amended to read:

> "In those cases when genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations. (See Section IV–E–1–b–2–f)."

B. A new section IV–E–1–b–2–(f) would be added to the Guidelines as follows:

> "IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes. (See Section III–B–3)."

An alternate amendment of the second paragraph of Section III–B–3 of the Guidelines has been proposed by Dr. Irving Johnson of Lilly Research Laboratories, as follows (amended language in italics):

> "In those cases where genetic exchange has not been demonstrated between two bacterial species A and B neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under *physical containment and conditions specified by the local IBC based on its consideration of the biological properties of the organisms.*"

7. *Request to Delete Language Requiring the use of Cauliflower Mosaic Virus (CaMV) mutants lacking the Aphid Transmission Factor from Section III–C–3. Request for Permission to Transfer DNA fragments from Aphid Transmissible to Aphid Nontransmissible CaMV.* Dr. Robert J. Shepherd of the University of California at Davis has requested that the following paragraph be deleted from section III–C–3 of the Guidelines:

> "The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid transmission factor."

In addition, Dr. Shepherd requests that he be permitted to transfer DNA segments from aphid transmissible to non-aphid transmissible strains of cauliflower mosaic virus in order to study the factors determining aphid transmissibility. This experiment could be considered as being prohibited under Section I–D–3 of the Guidelines. Dr. Shepherd suggests that it is inconsistent to restrict studies with cauliflower mosaic virus to non-insect transmissible strains when no such restriction is imposed on studies with bean golden mosaic and related viruses, the only other known group of DNA plant viruses. Dr. Shepherd suggests that under the P1 containment conditions specified in the Guidelines, cauliflower mosaic virus is easily confined to a growth chamber or greenhouse. In addition, the virus has a very restricted host range and is neither seed nor pollen transmitted, providing even further containment.

8. *Proposal to Amend Section III–C–4 of the Guidelines.* Dr. Milton Zeitlin of Cornell University proposed that Section III–C–4 of the Guidelines be amended to redefine a P1 greenhouse or growth chamber when certain infectious agents other than viruses are used as vectors. He proposes that the following

language should be added at the end of III-C-4:

> "and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules."

Dr. Zaitlin notes that a P1 greenhouse or growth chamber is described in Section III-C-3, Plant Viral Host-Vector Systems, in which there is a recommendation for positive air pressure in order to exclude insects which might transmit viruses. However, in the conditions considered in Section III-C-4, i.e. Plant Host-Vector Systems other than Viruses, the propagules themselves (spores, mycelia, etc.) should be contained, and a negative air pressure would be more appropriate.

9. *Cloning of Foot and Mouth Disease Virus—Stage II.* At the December 6-7, 1979 meeting, the RAC considered a proposal submitted by investigators at the Plum Island Animal Disease Center to permit the cloning of foot and and mouth disease virus (FMDV) in an *E. coli* K-12 host-vector system. Since FMDV virus is classified as a Class 5 agent, an exception to a prohibition of the Guidelines (I-D-1) was requested for cloning segments of the virus. Stage I of the proposal involves the cloning of double-stranded cDNA from RNA isolated from the virus. Subgenomic fragments of the viral genome are to be propagated in an *E. coli* K-12 host employing plasmid pBR322 as a vector. Clones containing FMDV sequences of interest would be analyzed and collected. The Director, NIH on recommendation of the RAC, approved on January 17, 1980 the formation of recombinants between fragments of FMDV and plasmid pBR322 as outlined in Stage I of the proposal. This stage is being carried out at the Plum Island Animal Disease Center.

The second stage of the scientific plan is proposed to be carried out in a joint effort at the laboratories of Genentech, Inc., South San Francisco, California. After clones containing viral sequences of interest have been assayed for lack of infectivity, they would be transferred to Genentech, Inc. for detailed molecular characterization. An attempt would be made to modify clones to produce polypeptides of interests, specifically the VP_1 polypeptide.

The RAC at its December 6-7, 1979 meeting recommended that a Working Group of the RAC be formed to report back to the full RAC after examination of the testing data on the infectivity of the clones produced at Plum Island under Stage I, prior to their removal from Plum Island. The Director, NIH accepted this recommendation on January 17, 1980. As Stage I of the project is nearing completion, the investigators now propose to submit testing data on the infectivity of the clones to the RAC Working Group and the RAC at its June 5-6 meeting in order to seek approval for further stages of the project. The RAC will consider whether they recommend that the clones may be removed from Plum Island, and what they judge to be the appropriate containment level for work with the clones at Genentech, Inc.

10. *Request for an Exception to a Prohibition: Field Testing of Corn Genes Put into Corn (Zea mays) with an E. coli or S. cerevisiae Vector.* Dr. Ronald Davis of Stanford University Medical Center has requested permission from the RAC to field test corn plants (Zea mays) which have been transformed by corn DNA or modified corn sequences cloned in *Escherichia coli* or *Saccharomyces cerevisiae* host-vector systems. Approval of this request requires an exception to Prohibition I-D-4, which prohibits deliberate release into the environment of any organism containing recombinant DNA.

11. *Request for Certification of a Bacillus subtilis Strain as the Host Component of an HV2 Host-Vector System.* Dr. William F. Burke, Jr. of Arizona State University requests the RAC to certify *Bacillus subtilis* strain ASB298 as the host component of an HV2 host-vector system. Dr. Burke has supplied ORDA with a report outlining the construction and characteristics of this strain.

12. *Proposed Revision of Section III-C-1-e.* A notice appeared in the Federal Register of January 31, 1980 concerning proposed revision of Section III-C-1-e, and its subsections. It was recommended that Sections III-C-1-e, III-C-1-e-(1), III-C-1-e-(1)-(a), and III-C-1-e(1)-(b) of the Guidelines be changed and that a new Section III-C-1-e-(1)-(c) be added. Section III-C-1-e-(2) would remain unchanged. The RAC, at its March 6-7, 1980 meeting, recommended adoption of Sections III-C-1-e, III-C-1-e-(1), and III-C-1-e-(1)-(a) as published in the Federal Register of January 31, 1980, with certain modifications in Section III-C-1-e-(1)-(a).

The Director, NIH, accepted this recommendation and promulgated the following sections in the Federal Register of April 11, 1980:

> "III-C-1-e. All Viral Vectors.
>
> III-C-1-e-(1). Other experiments involving eukaryotic virus vectors can be done as follows:
>
> III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

At its March 6-7, 1980 meeting, the RAC voted to defer consideration until the June 5-6, 1980 meeting of the new Sections III-C-1-e-(1)-(b) and III-C-1-e-(1)-(c) as proposed in the Federal Register of January 31, 1980, and requested that a working group develop additional information. An *ad hoc* working group will meet on May 13, 1980 during the meeting of the American Society for Microbiology. The report of the working group will be considered at the June 5-6, 1980 RAC meeting. Accordingly, the proposed revised Sections III-C-1-e-(1)-(b) and III-C-1-e-(1)-(c) are again published for public comment prior to consideration at the June 5-6, 1980 meeting:

> "III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the helper virus are not able to grow in human cells.
>
> III-C-1-e-(1)-(c). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P3 containment if wild type strains of the helper virus are able to grow in human cells."

13. *Lowering of Containment Levels for Characterized Clones or Purified DNAs by ICBs.* The December 1978 NIH Guidelines specified in Sections III-A-3-a, III-A-3-b, and IV-D-3-b, conditions under which the local Institutional Biosafety Committee could, without NIH prior approval, give approval for a single step reduction in physical or biological containment. When the Guidelines were revised in January 1980 and Section III-A was changed from covering experiments with the *E. coli* K-12 host-vector system to covering experiments with certain HV1 and HV2 systems, the ability of the IBC to grant such single step reductions without NIH approval was withdrawn. (This is discussed on page 69246 of the November 30, 1979 Federal Register). The RAC at its March 6-7, 1980 meeting voted 9 to 1 with 3 abstentions that there be published in the Federal Register for comment, and brought back to the RAC at its June 5-6, 1980 meeting, proposed revisions of the Guidelines to permit a single step reduction in physical or biological containment for characterized clones or purified DNA, to be approved by the local IBC, without prior NIH

approval. Accordingly the following changes in the Guidelines are proposed:

A. Section III–A–3–a is to be amended as follows:

> "III–A–3–a. *Purified DNA Other than Plasmids, Bacetriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may be decreased one step in physical containment (P4→P3; P3→P2; P2→P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3→HV2; HV2→HV1) while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1+HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)–(e).)"

B. Section III–A–3–b is to be amended as follows:

> "III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.
>
> Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1+HV1, or reduction of containment levels by more than step, which also require prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–3–(e).)"

C. Section IV–D–1–c–(3) is to be amended as follows:

> "IV–D–1–c–(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3)."

D. The Note in Section IV–D–3–b is to be amended as follows: (Additional language in Italics).

> "Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. *Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been purified and are judged to be free of harmful sequences (See Section III–A–3–a) and for (ii) clones that have been characterized and judged to be free of harmful sequences (see Section III–A–3–b).* It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion."

E. Section IV–E–1–b–(3)–(e) is to be amended as follows:

> "IV–E–1–b–(3)–(e). Lowering containment levels in certain cases for characterized clones or purified DNA (see Sections III–A–3–a and –b, and Footnotes 3 and 41);"

14. *Transfer of DNA Segments Cloned in Saccharomyces cerevisiae Host-Vector Systems to Higher Plants.* Dr. Jane Setlow of Brookhaven National Laboratory, has suggested that the Guidelines include a general provision for cloning plant DNA in *Saccharomyces cerevisiae* and subsequently returning the cloned DNA to the plant. She proposes that the first sentence of Section III–c–7–b be amended to read as follows:

> "III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under appropriate containment conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to Pl and appropriate to the organism under study [2A]."

15. *Status of Variola and Whitepox Viruses.* It was suggested at the RAC March 6–7, 1980 meeting that any mention of the variola viruses in the Guidelines be marked with a symbol so that reference could be made to world organization policy regarding these viruses and worldwide efforts at eradication.

Accordingly, it is proposed that Appendix B, Classification of Microorganisms on the Basis of Hazard, be amended so that each mention of Alastrim, Smallpox and Whitepox will be marked with a symbol. A footnote at the bottom of the page bearing this symbol will read:

> "All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta)."

16. *Request for Consideration of Appropriate Containment Conditions.* Dr. Paul S. Sypherd of the University of California, Irvine has requested consideration of the containment levels appropriate to the return of *Mucor racemosus* DNA cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus.* In addition, Dr. Sypherd requests permission to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned yeast sequences.

17. *Proposal to Amend Section I–D–6 of the Guidelines.* Dr. Irving Johnson of Lilly Research Laboratories, proposes that Section I–D–6 of the Guidelines be amended, as follows:

A. Delete the second paragraph of Section I–D–6 which currently reads as follows:

> "We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale."

B. Amend the third paragraph of Section I–D–6 to read as follows (amended language in italics):

> "Experiments in Categories I–D–1 to I–D–6 may be excepted (4) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(e).) *Those experiments utilizing an E. coli K–12 host-vector system and meeting the containment requirements of Section III–O are excluded from these prohibitions.*"

18. *Proposals to Amend Several Sections of Part IV, Roles and Responsibilities.* Dr. Irving Johnson of Lilly Research Laboratories, has proposed several amendments of Part IV of the Guidelines, "Roles and Responsibilities," as follows:

A. Amend Section IV–E–1–b–(3)–(d) under Section IV–E–1–b, *"Specific Responsibilities of the Director, NIH,"* to read as follows (new language in italics):

> "IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6).
>
> *Further volume increases at the approved containment level, e.g., for industrial development and production purposes, of previously approved (by the Director of NIH) large-scale experiments may be subsequently approved by the local IBC in accordance with Section IV–D–3–a. The IBC will notify ORDA of such volume increases.*"

B. Amend the second paragraph of Section IV–E–2, *Recombinant Advisory Committee,* to read as follows (amended language in italics):

"The members of the committee shall be chosen to provide, collectively, expertise in scienctific fields relevant to recombinant DNA technology and biological safety—e.g. microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. *Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production.* At least 20 percent of the members shall be persons knowledgeable in an applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as non-voting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities."

The first and third paragraphs of Section IV–E–2 would remain unchanged.

C. Amend Section IV–E–2, *Recombinant Advisory Committee,* to add a new section IV–E–2–b, as follows:

"IV–E–2–b. A permanent subcommittee of the RAC shall be responsible for advising the Director of NIH, on the actions, listed in Section IV–E–1–b–(3)–(d) pertaining to large-scale applications. Submissions that are in compliance with the Guidelines may be recommended by the subcommittee to the Director of NIH for approval. The subcommittee shall also be authorized to consider preliminary plans for large-scale operations and to recommend approval contingent upon completion of the large-scale facility according to those plans. The subcommittee will be responsible for expeditiously processing applications."

19. *Request for Use of Conjugative Plasmids and Transducing Phages in an Escherichia coli K–12 Host.* Dr. A. I. Bukhari of the Cold Spring Harbor Laboratory has requested permission from the RAC to employ conjugative plasmids or transducing phages in recombinant DNA experiments involving *Escherichia coli* DNA when a small fragment of heterologous DNA is used as a linker. In the specific request, Dr. Bukhari requests approval to use a fragment of Ad₂ DNA as linker DNA.

20. *Request for Exception to a Prohibition to Clone DNA from a Class 3 CDC Agent.* Dr. Sebastiao Baeta Henriques of the Instituto de Ciencias Biologicas, Belo Horizonte, Brazil, requests the RAC to approve an exception to a prohibition to clone genes from *Schistosoma mansoni,* a Class 3 CDC agent, in *E. coli* K–12. This request was considered briefly at the March 6–7, 1980 RAC meeting. Dr. Henriques was requested to provide information as to why he is requesting NIH approval for a non-NIH project to be conducted in Brazil. Dr. Henriques has provided NIH with an additional letter of explanation.

Additional Announcements of the Director, NIH

Section IV–E–1–b–(3)–(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences." Accordingly, several requests for authorization to culture, on a large scale, recombinant DNA host-vestor systems have been received and reviewed by the NIH.

I. Genentech, Inc.

On the recommendation of the RAC, the following requests from Genentech, Inc. have been approved by the Director, NIH:

A. On April 1, 1980, the Director, NIH approved a request from Genentech, Inc. for the large-scale culture up to 750 liters of EK1 host-vector systems containing a plasmid into which has been ligated a combination of chemically synthesized DNA and cloned cDNA coding for human growth hormone.

B. On April 9, 1980, the Director, NIH approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 host-vector systems constructed to produce various chimeric fusion proteins containing either somatostatin, human insulin A chain, human insulin B chain, human proinsulin, and thymosin alpha1.

Both requests were approved with the understanding that Genetech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site, and to certain additional stipulations.

The principal investigators are Drs. Michael Ross and Norm S. C. Lin. The work is to be done, as stipulated by the Genentech, Inc. submission, in a "P2 laboratory housing fermentors modified and tested to totally contain the recombinant organisms until they are chemically or physically killed at the end of each fermentation * * * at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080."

II. Eli Lilly & Co.

A. On April 7, 1980, the Director, NIH, approved a request from Eli Lilly and Company for large scale culture of an EK1 host-vector system containing a plasmid coding for human proinsulin covalently joined to *E. coli* β-galactosidase, at the P2 and P1–LS levels of containment.

B. On April 7, 1980, the Director, NIH, approved a request to amend a previous submission of Eli Lilly and Company. In the previous submission, which was approved on October 5, 1979 by the Director, NIH, Eli Lilly and Company requested permission to culture on a large-scale EK1 host-vector systems separately carrying the chemically synthesized insulin A chain and B chain. The February 1980 submission requested permission to amend this protocol to permit culture of the same host-vector systems in larger volumes at the P1–LS level of containment.

C. On April 9, 1980, the Director, NIH approved a joint request from Eli Lilly and Company and Genentech, Inc., for large scale culture of EK1 host-vector systems producing human proinsulin, insulin A chain, and insulin B chain as chimeric fusion proteins in RAC-approved fermentors of Eli Lilly and Company at the P2 and P1–LS levels of containment.

These requests were approved with the understanding that Lilly Research Laboratories have agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigator for these projects is Dr. Lawrence E. Day. The large scale growth of the organisms is to be carried out at plant facilities located at 1200 South Kentucky Avenue, Indianapolis, Indiana 46206.

Dated: April 23, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

JUNE 5-6, 1980

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

JUNE 5-6, 1980

The Recombinant DNA Advisory Committee (RAC) was convened for its nineteenth meeting at 9 a.m. on June 5, 1980, in Conference Room 6, Building 31C, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. Dr. Jane K. Setlow (Chairman), Biologist, Brookhaven National Laboratory, presided. In accordance with Public Law 92-463 the meeting was open to the public, except for the review of proposals involving proprietary information as the last item of business on June 6, 1980.

Committee members present for all or part of the meeting were:

Dr. Abdul Karim Ahmed; Dr. David Baltimore; Dr. Kenneth Berns, Dr. Winston Brill; Dr. Francis Broadbent; Dr. Allan Campbell; Mrs. Zelma Cason; Dr. Richard Goldstein; Dr. Susan Gottesman; Ms. Patricia King; Dr. Sheldon Krimsky; Dr. Werner Maas; Dr. Elena Nightingale; Dr. Richard Novick; Dr. David Parkinson; Mr. Ray Thornton; Dr. LeRoy Walters; Dr. Luther Williams; Dr. Frank Young; Dr. Milton Zaitlin; and Dr. William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Mr. Glenwood Achorn, Higdon, Arkansas; Dr. Gerald Fink, Cornell University, Ithaca, New York; Dr. Elmer Gaden, University of Virginia, Charlottesville, Virginia; Dr. Myron Levine, University of Maryland School of Medicine, Baltimore, Maryland; Dr. Gerard McGarrity, Institute for Medical Research, Camden, New Jersey; Dr. Robert McKinney, Enviro Control, Inc., Rockville, Maryland; and Dr. John Scandalios, North Carolina State University, North Carolina.

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[1] The RAC is advisory to the NIH, and its recommendations should not be considered as final and accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

The following non-voting members and liaison representatives were present:

Dr. Charlotte Bell, U. S. Department of Justice; Dr. Walter R. Dowdle, Center for Disease Control; Dr. George Duda, U. S. Department of Energy; Dr. George Gordon, U. S. Department of Commerce; Dr. Richard Hedrich, National Endowment for the Humanities; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Tetsuo Iino, University of Tokyo; Dr. Herman Lewis, National Science Foundation; Dr. David Logan, U. S. Department of Labor; Mr. Melvin Myers, National Institute for Occupational Safety and Health; Dr. Jane Schultz, U. S. Veterans Administration; Dr. Sue Tolin, U. S. Department of Agriculture; and Dr. William J. Walsh, III, U. S. Department of State.

Other National Institutes of Health staff present were:

Dr. Marilyn Bach, NIAID; Dr. Stanley Barban, NIAID; Dr. W. Emmett Barkley, ORS; Mrs. Betty Butler, NIAID; Dr. Robert J. Byrne, NIAID; Dr. Irving Delappe, NIAID; Dr. Donald S. Fredrickson, Director, NIH; Dr. Steve Havas, NHLBI; Dr. John Irwin, ORS; Dr. Richard Krause, NIAID; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Dr. Carl Rhodes, NIGMS; Mr. Marc Stern, OC-OD; Dr. Bernard Talbot, OD; Dr. Rudolf Wanner, ORS; Ms. N. Wender, OC-OD; Dr. Karl Western, NIAID; and Dr. Burke Zimmerman, OD.

Others in attendance for all or part of the meeting were:

Dr. John Adams, Pharmaceutical Manufacturers Association; Dr. Lucile Adamson, Occupational Safety and Health Administration; Dr. E. A. Agostini, Pfizer, Inc.; Dr. Howard Bachrach, U. S. Department of Agriculture; Dr. D. E. Baldwin, Schering Corporation; Dr. Barry Beyer, Schering Corporation; Dr. Peter Bostock, New Brunswick Scientific Company, Inc.; Dr. Richard Brock, Australian Embassy; Dr. William Can, Jack Raymond & Company; Mr. Bob Byrnes, Genentech, Inc.; Dr. Jerry Callis, U. S. Department of Agriculture; Dr. C. T. Chen, Occupational Safety and Health Administration; Mr. Jeffrey Christy, Blue Sheet; Dr. R. John Collins, University of California, Los Angeles; Dr. Aileen Compton, Smith-Kline; Dr. T. H. Fraser, Upjohn Company; Dr. Jeffrey Freedman, Office of General Council, DHHS; Dr. Leonard C. Gosule, Food and Drug Administration; Dr. Patricia Guerry, Genex Corporation; Dr. Lowell Harmison, Office of Assistant Secretary for Health; Dr. Zsolt Harsanyi, Office of Technology Assessment; Dr. Paul Hung, Abbott Research Laboratories; Dr. James Hunt, Chemapec; Dr. Evelyn Hurlburt, Johns Hopkins School of Hygiene; Dr. Dorothy Jessup, U. S. Department of Agriculture; Dr. Irving Johnson, Eli Lilly & Company; Dr. Attila I. Kadar, Food and Drug Administration; Mr. Alan Kaplan, Attorney, Washington, D. C.; Dr. Geoffrey Karney, Office of Technology Assessment; Dr. Paul Kaufman, Pharmaceutical Manufacturers Association; Dr. Paul Leibowitz, Schering Corporation; Ms. Carter Leonard, Blue Sheet; Dr. M. A. Levine, Environmental Protection Agency; Dr. Dan Liberman, Massachusetts Institute of Technology; Dr. James McCullough, Library of Congress; Dr. T. McKillop, ICI Pharmaceuticals; Dr. J. Metcalfe, Environmental Protection Agency; Dr. Henry I. Miller, Food and Drug Administration; Dr. DeLill Nasser, National Science Foundation; Dr. Ann Norberg, Monsanto Company; Mr. Tom O'Brien, Subcommittee on Science, Research and Technology, U. S. House of Representatives; Dr. Kim Olson, Genentech, Inc.; Mr. Seth Pauker, National Institute for Occupational Safety and Health; Dr. William Pilacinski, Molecular Genetics, Inc.; Dr. W. P. Quill, Office of Technology Assessment; Mr. Richard Riseberg, Office of General Council, DHHS; Dr. Michael Ross, Genentech, Inc.; Dr. A. H. Schaver, Competitive Research Grants Office, USDA; Mr. Harold Schmeck, New York Times; Dr. Brian Sheehan, Consultant; Dr. Mark Shon, Genex Corporation; Dr. Nowell Stebbing, Genentech, Inc.; Dr. Gerald Still, U. S. Department of Agriculture; Dr. Robert Swanson, Genentech, Inc.; Mr. Jeff Swarz, Teknekron, Inc.; Dr. Susan Walton, Bioscience; Dr. Charles Weiner, Massachusetts Institute of Technology; Dr. Marvin Weinstein, Schering Corporation; Dr. Susan Wright, University of Michigan; and Dr. Robert Zaugg, U. S. Senate Commerce Committee.

I. CALL TO ORDER AND OPENING REMARKS

Dr. Jane Setlow, Chairperson, called the meeting to order at 9:00 a.m., March 5, 1980, and introduced Dr. Donald S. Fredrickson, Director, National Institutes of Health (NIH).

Dr. Fredrickson noted that the terms of several members of the RAC were terminating. These members are: Dr. Samuel Proctor, Professor of Education, Rutgers University; Dr. Milton Zaitlin, Professor of Plant Pathology, Cornell University; Dr. LeRoy Walters, Director, Center for Biothics, Kennedy Institute, Georgetown University; Dr. Francis Broadbent, Professor of Soil Microbiology, University of California, Davis; and Dr. Jane Setlow, Brookhaven National Laboratory, the current Chairperson of the RAC. Dr. Fredrickson said two members have been reappointed for an additional two years: Dr. Ramon Pinon, and Dr. Frank Young. Dr. Fredrickson awarded certificates of service to the five retiring members and thanked them for their service.

Dr. Fredrickson said that five new members will begin their terms on July 1, 1980. These new members are: Dr. Gerard McGarrity, Head, Department of Microbiology, Institute for Medical Research, Camden, New Jersey; Dr. Myron M. Levine, Director, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore; Dr. John G. Scandalios, Head, Department of Genetics, North Carolina State University, Raleigh; Dr. Nina Federoff, Staff Member, Carnegie Institution of Washington, Baltimore, Maryland; and Dr. King Holmes, Head, Division of Infectious Diseases, Public Health Service Hospital, Seattle, Washington. Drs. McGarrity, Levine, and Scandalios were present at the June 5-6, 1980 meeting and were introduced by Dr. Fredrickson.

Dr. Fredrickson announced the appointment of Mr. Ray Thornton as the Chairperson of the RAC effective July 1, 1980. Dr. Fredrickson noted that Mr. Thornton is President designate of Arkansas State University. He wished Mr. Thornton success in both these roles. Dr. Setlow introduced Dr. Iino of the University of Tokyo, Japan, a liaison representative to the RAC.

II. VOLUNTARY COMPLIANCE

Dr. Fredrickson addressed the following statement on voluntary compliance to the RAC:

"I am aware that the RAC has engaged in much discussion of the task it has accepted to review applications involving proprietary information. Usually, these are submitted by private concerns not supported by federal funds and in voluntary compliance with the NIH Guidelines. Particularly, some members of the RAC have chafed under the need for examination and approval of scale-up operations in industry, a result of the 10-liter limit in the Guidelines. Certainly, most members find the task onerous and not a few still find distasteful the scientific review of information which is subject to safeguards including criminal penalties for violation. Some members of the RAC also are of the opinion:

1. that the task involves judgments about apparatus for physical containment which do not rest upon the same experience and training useful for decisions about molecular biology and other aspects of recombinant technology; or
2. that when the judgments about physical containment require site inspections in industry, such visits belie the insistence of NIH, and its Director, that this agency eschews a regulatory role;
3. and several members believe consideration of any part of an application from the private sector, perhaps even any proprietary data, should be done only by a regulatory agency, which NIH is not.

I am grateful to all the members of the RAC, whatever their views on this subject, for subordinating their feelings to the extent that they have continued to carry out these important duties. In so doing, they have made it possible for all users of recombinant technology in the U.S.A. to do so under two important conditions that obtain in nearly all other advanced countries of the world. These are:

1. the determination of appropriate procedures by a single national body, responsible for interpreting and developing uniform national guidelines, and

2. the regulation of a rapidly evolving science without passage of special statutes, avoiding inflexibility often attendant upon such legislation.

These important conditions are among those necessary for maintenance of parity among the nations for safe use of this technology and access to its benefits. It is essential that the RAC, whose deliberations affect the course of this field of science throughout so much of the world, not alter these conditions by precipitous changes in its procedures.

Nevertheless, I am sympathetic to this malaise of the RAC. We must try to relieve it--and to move NIH back from what some see as a position on the brink of regulatory involvement. In thinking about such relief, I note especially the rising interests of OSHA and NIOSH in ascertaining any threats to workers inherent in industrial scale-ups. FDA and other regulatory agencies have also begun to test their reflexes in a similar way. FDA sponsored here this week a useful meeting on industrial application of recombination for production of biologics.

It is appropriate that these agencies begin to consider their mandates as industrial use of recombinant techniques grows. One of the earliest tasks of the Federal Interagency Advisory Committee on Recombinant DNA Research (IAC) was an analysis of the authorities of the regulatory agencies in this area. The results indicated that no single agency has clear authority over laboratory research, but there obviously were appropriate roles for such agencies in the oversight and regulation of industrial-scale use of recombinant methodologies. All the regulatory agencies also have continuously been kept fully informed of the activities concerning the NIH Guidelines, through their membership on the IAC, and through their liaison membership on the RAC.

At the same time, it should be stated here with candor that no federal regulatory agency presently has anything like the in-house competence to perform--for industrial applications--all of the present tasks of the RAC. Development of such competence will require time and expense, and it would not arise suddenly upon the passage of any statute. Furthermore, we are still at a stage where the creation of more than one RAC in this country--one for industry, one for non-profit research--would be inefficient and destroy the unity of information-sharing and decision-making about the many aspects of handling recombinant DNA activities that facilitate appropriate evolution of guidelines.

It is, however, apparent that in taking any actions appearing to validate physical containment on an industrial scale, the NIH also has insufficient in-house competence, and, like the RAC, must rely on consultants. In these determinations, a regulatory agency like

OSHA, or its research partner, NIOSH, can use consultants as effectively as can NIH, and may have more staff competence. Such judgments can more appropriately be carried out by them, especially when they are related to a regulatory mission.

From these considerations, I believe that the RAC should consider at least two options as it deliberates upon its procedures for handling industrial or private requests:

1. to continue as it is until more experience dictates some new mode. Here keep in mind that an Industrial Practices Subcommittee of the IAC is actively studying the problem and will report to the IAC in the fall;

2. to restrict its review and decisions to questions concerning biology and related issues of laboratory containment; and when containment for large-scale use is the issue, to judge only whether the specified P-LS level is appropriate (referring to the model designations in the April 11, 1980 Federal Register). The regulatory agencies will be fully apprised of such actions. They can make on-site evaluations if they deem them to be appropriate to their regulatory mandate.

With regard to the second option, I would note here that past actions of the RAC have included the insertion of a provision for visits by an NIH-designated person to industrial facilities that have been the subject of a RAC decision. If such visits are for purposes of correlating scientific information relevant to the duties of the RAC, they would seem to me to be still appropriate. Inspections for regulatory purposes shall be carried out by OSHA/NIOSH or other regulatory agencies having relevant authority.

Finally, let me emphasize that it would not be appropriate for the RAC to decide, in exasperation, that it would like to avoid handling any proprietary data. From time-to-time, such material will have to be evaluated by the RAC in pursuit of its duties. I welcome the opportunity to participate in discussion of this matter to the extent that my presence will be useful."

Following his remarks, Dr. Fredrickson opened the floor to discussion. Dr. Walters asked Dr. Fredrickson to amplify his remarks on the types of site visits which might be made to an industrial installation.

Dr. Fredrickson replied that a visit to gather information regarding the implementation of RAC decisions and for the purpose of discerning how these decisions were applied to large-scale work was different from a visit by a regulatory agency. A visit from a regulatory agency would be made with the express purpose of evaluating compliance with the law and protection of employees.

Dr. Fredrickson said that a proposed alternative method of reviewing industrial applications, where subsequent to the RAC setting a P-LS level of biological containment, the Director, NIH, would select a panel of consultants for the purpose of evaluating the details of a company's physical containment is, in his opinion, not appropriate.

Dr. Parkinson said that state OSHA authority extends to university laboratories as well as to industrial workplaces. He said he saw no point in visiting a work place if worker safety is not evaluated. Dr. Krimsky asked Dr. Fredrickson whether the NIH has a role, under the voluntary compliance program, in establishing more rigorous standards of keeping health data in industries. Dr. Fredrickson replied that the Industrial Practices Subcommittee of the Interagency Committee is considering this issue.

Following Dr. Fredrickson's departure, Dr. Gartland reviewed the history of the NIH voluntary compliance program. He said that at the time the December 1978 NIH Guidelines were issued, the Food and Drug Administration (FDA) announced its intent to propose regulations for recombinant DNA research in the private sector. In February 1979, the RAC unanimously approved interim procedures for reviewing large-scale applications. In May 1979, the RAC was briefed on procedures for the protection of proprietary information, and a working group was established to examine physical containment requirements for large-scale experiments. Responses to the FDA's notice of intent to propose regulations questioned the agency's legal authority to regulate in this area. In view of these comments, the Director, NIH, and the Commissioner, FDA, developed a draft supplement to the NIH Guidelines that would extend the Guidelines on a voluntary basis to industry. On July 16, 1979, the Federal Interagency Advisory Committee on Recombinant DNA Research, which includes all relevant federal regulatory and research agencies, unanimously agreed that NIH should publish for comment this proposed supplement to the Guidelines. In September 1979, the RAC recommended incorporation of the supplement into the Guidelines. The supplement was then published in the November 30, 1979, Federal Register for thirty days of additional comment. The revised NIH Guidelines, promulgated in January 1980, incorporated the supplement as Part Six, "Voluntary Compliance." The RAC, at the September 1979 meeting, also accepted a report of the working group on large-scale procedures. This report specified application procedures for large-scale experiments, including the types of information to be submitted for review, and a statement regarding confidentiality. In September 1979, the RAC went into the first closed session to review proprietary information. In December 1979, the RAC agreed to review the voluntary compliance program at its June 1980 meeting. Draft guidelines for large-scale physical containment were also reviewed. The Federal Interagency Advisory Committee, in February 1980, recommended the establishment of an Industrial Practices Subcommittee to consider what steps, if any, beyond the NIH Guidelines, need to be taken regarding occupational health and industrial production in recombinant DNA technology. The RAC, at its March 1980 meeting, recommended that the large-scale physical containment recommendations be published in the Federal Register.

Dr. Gartland said that to date, thirteen institutions not otherwise covered by the NIH Guidelines have registered their biosafety committees with the NIH. Seven proposals to exceed the ten-liter limit have been recommended for approval by the RAC and have been approved by the NIH. These proposals have been voluntarily submitted by two companies, Eli Lilly and Company, and Genentech, Inc.

Dr. Gottesman proposed that for large-scale experiments, the RAC continue to determine whether a clone is fully characterized and free of harmful sequences and that it set a P-LS level. However, the RAC would not evaluate the physical containment facilities. Dr. Gottesman said that this responsibility would be shifted to the companies themselves.

Dr. Nightingale supported Dr. Gottesman's position but said she would prefer receiving some feedback of information to the RAC on implementation of RAC decisions, at least on an interim basis. Dr. Baltimore stated that Dr. Gottesman's proposal would allow companies to continue on in larger and larger volumes without rechecking with the RAC, once they had received initial RAC approval to exceed 10 liters, based on a RAC determination that a clone is fully characterized and free of harmful sequences. Dr. Baltimore suggested that in adopting Dr. Gottesman's proposal, the RAC would be abrogating an area of self-imposed responsibility, leaving no oversight in any agency or group.

Dr. Irving Johnson noted that Eli Lilly and Company had already been visited by representatives of the RAC, National Institute for Occupational Safety and Health (NIOSH), and the Food and Drug Administration (FDA). Dr. Walters said he was sympathetic to the concept of distinguishing between evaluating biological aspects and evaluating physical containment. He suggested that perhaps a RAC subcommittee, modeled after the British Genetic Manipulation Advisory Group (GMAG) subcommittee, might be constituted, and might make site visits to industry to verify that the equipment is as described. Dr. Young said that there should be increased dialogue between ORDA and companies on containment facilities if RAC is not going to evaluate facilities on a case-by-case basis.

Dr. Goldstein asked whether facilities where large volumes of etiological agents were grown in non-recombinant DNA cases, e.g., for vaccine production, were inspected and monitored. Dr. Dowdle, of the Center for Disease Control (CDC), said that the Public Health Service has issued guidelines regarding the safe handling of infectious agents. Dr. Baltimore asked if these guidelines have statutory authority. Dr. Dowdle responded that the guidelines have no statutory authority and compliance is voluntary, but experience has shown that guidelines become an effective accepted standard.

Ms. King said that she was troubled by a compliance system where only some aspects of a situation are reviewed. She was basically opposed to certifying an application when no adequate means of ensuring compliance exists. She felt the RAC does not have the legal authority to regulate industry and said voluntary compliance, in her experience, is not a viable oversight mechanism. She said, however, she found Dr. Gottesman's proposal preferable to the current situation.

Dr. Campbell said he supported Dr. Gottesman's proposal. However, he shared the concerns that Dr. Baltimore voiced about infinite scale-up. He suggested that any recombinant organism that is propagated in very large volumes should be subjected to some type of testing in terms of toxicity, antigenicity, etc.

Mr. Thornton said that by action of the RAC, and by approval by the Director, NIH, the charge to the RAC now includes oversight of a program of voluntary compliance. He stated that the U.S. system of government is based upon consent and that consent to be bound by the NIH Guidelines has been expressed. He hoped that changes from the current situation would be done by a orderly process.

Dr. Novick said he would be comfortable supporting Dr. Gottesman's proposal if the RAC would go on record as endorsing, in principle, the bill introduced by Senator Stevenson's, S. 2234. Ms. King noted that the RAC had gone on record as supporting the concept of mandatory compliance.

Dr. Young suggested that the RAC might adopt language indicating that in the recommendation of scientific feasibility and levels of physical containment, the action of the RAC should not be construed as an industrial certification.

Dr. Baltimore said that Dr. Gottesman's proposal, in the long term, would be an appropriate role for the RAC. However, he expressed concern that adoption of Dr. Gottesman's proposal, at the present time, would create a vacuum of oversight. Therefore, he proposed that Dr. Gottesman's proposal be forwarded to the Interagency Committee as RAC's view of a long-term goal. Ms. King said she shared Dr. Baltimore's concern for orderly process, but felt the RAC should begin definite orderly action towards its goal.

Dr. Goldstein said that approximately a year ago the RAC indicated to the Director, NIH, that the RAC was uncertain of its role in voluntary compliance, and requested that other federal agencies become involved in this area. He said that the Federal Interagency Committee has convened several times since then, but has made no concrete proposals. Dr. Goldstein said the RAC should not continue in its present mode. He suggested that the surest mechanism to have the Interagency Committee assume responsibility is for RAC to disclaim any further responsibility. Dr. Baltimore said he did not believe that the Interagency Committee has the authority to take action itself. Ultimately, it is the Occupational Safety and Health Administration (OSHA) which could take action. Dr. Baltimore speculated that the minimum time to institute OSHA action would be a year. Dr. Baltimore asked NIOSH and OSHA representatives to estimate the time required. Dr. Logan of OSHA said that OSHA has not been particularly involved in this area in the ten years since OSHA was established. He said the agency recognizes the importance of this issue and is currently exploring approaches to the problem.

Dr. Goldstein suggested that the institution of a good medical surveillance program might allay many of the concerns expressed by the members of the committee. He suggested that Dr. Gottesman's proposal be amended to indicate that the RAC finds such a program desirable, and requests NIOSH and OSHA to implement a medical surveillance program. Dr. Gottesman suggested that a straw vote be taken to determine if the committee views her proposal as an appropriate end result before discussing any amendments or methods of implementation. She would then compose more precise language and the RAC might resume the discussion during the following day's agenda. She moved that the RAC take a straw vote on the following procedure for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA: "The RAC will determine whether a particular recombinant DNA containing strain is fully characterized and free of harmful sequences. Such a determination shall include specification of a containment level (P-LS level). Adherence to the specified containment conditions is the responsibility of the local IBC and appropriate regulatory agencies."

Dr. Baltimore preferred to defer the discussion to permit an ad hoc group to compose appropriate language. Ms. King stated that the RAC could vote in principle on Dr. Gottesman's proposal, and develop specific language later. Dr. Goldstein said that Dr. Baltimore's proposal would maintain the status quo for the time being and he would like to table the motion. Dr. Goldstein stated his belief that setting a deadline after which the RAC will no longer review industrial practices is the most effective means of having other agencies assume oversight responsibility.

Dr. Gottesman then repeated her motion. Dr. Krimsky proposed an amendment to Dr. Gottesman's motion, stating that the committee supports, (1) the principle that a health surveillance system be established, and (2) legislation that would require mandatory compliance for all institutions involved with recombinant DNA activities. Dr. Goldstein seconded Dr. Krimsky's proposed amendment. Dr. Gottesman said that Dr. Krimsky's amendment addressed issues quite different from those she wished to address, and said she would not accept the amendment. Dr. Young supported Dr. Gottesman's position. Dr. Krimsky then moved a substitute motion incorporating both Dr. Gottesman's language and his own proposed amendments. Dr. Setlow called the question on Dr. Krimsky's substitute motion. The RAC denied the motion by a vote of three in favor, thirteen opposed, and two abstentions.

Dr. Gottesman asked for a vote on her motion, and reread the wording of the motion:

> "It is the sense of the Committee that the following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. RAC will determine if a given recombinant DNA-containing strain is in fact 'fully characterized and free of harmful sequences.' Such a determination shall include specification of a containment level (P-LS level). Adherence to the specified containment conditions is the responsibility of the local IBC and appropriate regulatory agencies."

The RAC supported Dr. Gottesman's proposal by a straw vote of sixteen in favor, one opposed, and one abstention.

Dr. Young requested that two additional issues be addressed: (1) that RAC members be included on the Industrial Practices Subcommittee of the Federal Interagency Committee to facilitate both communication and action, and (2) that language be added to the recommended approvals of industrial applications to indicate that the action of the RAC should not be construed as certification of safe laboratory practices for scale-up. It was pointed out that the Interagency Committee is composed only of federal employees, and that the character of the Interagency Committee would be altered were Dr. Young's motion accepted. Dr. Young then withdrew his proposal.

Dr. Baltimore moved that Dr. Gottesman's motion be forwarded to the Federal Interagency Committee as the sense of the RAC's position, rather than taking precipitous action. He felt implementation of regulation by other agencies would take time and he preferred to see the RAC fill the vacuum until other agencies implement procedures.

Ms. King opposed Dr. Baltimore's motion. She said the RAC should implement actions which the members feel are best for the RAC. Dr. Baltimore reiterated his belief that adoption of an expiration deadline would leave aspects of industrial recombinant DNA activities, currently evaluated by RAC, without surveillance. Dr. Setlow called the question. The RAC denied Dr. Baltimore's motion by a vote of six in favor, ten opposed, and two abstentions.

Ms. King moved that the principles concerning voluntary compliance approved by the RAC today, including any modifications voted tomorrow, be given to ORDA for analysis of how they can be implemented consistent with the Guidelines. ORDA should take the necessary steps, including publication of language in the Federal Register, to permit the RAC to vote on implementation of these principles at the September 1980 meeting. Dr. Novick asked Ms. King if the sense of the committee proposal should be forwarded to the Interagency Committee for its consideration. Ms. King said she was willing to include this suggestion in her motion. The RAC accepted Ms. King's motion by a vote of fourteen in favor, none opposed, and four abstentions. There was further discussion of this issue on June 6, 1980 (see Section XXV of the Minutes).

III. MINUTES OF MARCH 6-7, 1980 MEETING

The RAC reviewed the minutes of the March 6-7, 1980 meeting (tab 902). Mr. Thornton said he had found three substantive errors and suggested several minor editorial changes. Dr. Walters suggested that the paragraph describing the Eli Lilly and Company facility be clarified. He also proposed a modification of the report of Dr. Emmett Barkley's statement. Dr. Walters read his modified language. Mr. Thornton concurred with Dr. Walters' suggestions and moved acceptance of the minutes with the suggested modifications. Dr. Young said he would suggest several minor corrections to ORDA. With these corrections, the minutes were approved unanimously with one member abstaining.

IV. REPORT OF RISK ASSESSMENT MEETING

Dr. Krause said that the National Institute of Allergy and Infectious Diseases (NIAID), has let a contract to develop a comprehensive training course on microbiological principles and safety practices. This course will be developed in accord with the recommendations of the American Society for Microbiology (ASM). He noted that the ASM suggested that training in all areas of microbiology was necessary and that recombinant DNA techniques are simply a subcategory of microbiological research.

Dr. Krause also said that NIAID would convene a meeting of Institutional Biosafety Committee (IBC) chairmen on November 24 and 25, 1980. He invited all RAC members to attend this meeting. This will be the first stage in an evaluation of the functioning of IBCs.

Dr. Krause then reported on the Risk Assessment Meeting held in Pasadena, California on April 5-6, 1980 (tab 896, 898, 898A). He said that 92 scientists with expertise in immunology, endocrinology, physiology, microbiology and infectious diseases attended. He said that the Pasadena meeting addressed two issues:

1. the potential direct adverse effects of hormone-producing or active polypeptide-producing strains of Escherichia coli K-12, and
2. the potential production of antibodies or autoreactive cells by individuals colonized by Escherichia coli K-12 elaborating eukaryotic polypeptides.

He said the workshop was to determine whether these two possibilities are valid scientific hypotheses, and if valid, to determine whether sufficient data existed to make a judgement concerning risk. If sufficient data were not available, the workshop was instructed to outline the studies necessary to develop an informational framework. He said that the majority of participants felt the issues were valid scientific questions, but in general, judged that risks from either of these scenarios were small. He said several recommendations were developed at the meeting: (1) it is desirable that E. coli cells carrying recombinant DNA and making a mammalian protein are introduced into living animals under conditions where they cause genuine infection (e.g., an abscess) with subsequent monitoring for breakdown of immune tolerance; (2) additional information is needed on the handling and absorption of polypeptides in normal and in pathological colon, and the potential effects of synthetic polypeptides on the bowel; and (3) additional information is needed on the frequency of plasmid transfer from Escherichia coli K-12 to anaerobes in the bowel. A fourth recommendation was that NIH communicate with CDC concerning health surveillance of workers using recombinant DNA technology.

Dr. Krause said that in order to address the first recommendation, NIAID will release either a Request for Proposals (RFP) or a Request for Applications (RFA) to solicit responses from interested investigators. To address the question of handling of polypeptides by normal and pathological colon and effects of synthetic polypeptides, Dr. Krause said that NIAID would cooperate with the National Institute of Arthritis, Metabolism, and Digestive Diseases to attempt to develop appropriate human studies. He said that studies currently funded by NIAID should provide information on the frequency of plasmid transfer from E. coli K-12 to anaerobes. He deferred discussion of implementation of the fourth recommendation to Dr. Dowdle of CDC, who was present at the RAC meeting. Dr. Dowdle said that consideration of this topic was included in the upcoming Federal Interagency Subcommittee meeting agenda.

Dr. Campbell said he concurred with Dr. Krause's summary of the Pasadena meeting. He added that workshop recommendations for studies in certain areas do not necessarily imply that such experiments are a necessary part of a risk assessment program; rather they may merely indicate that the experiment is feasible and would produce relevant knowledge.

Dr. Krimsky said that the Pasadena transcript seemed to reflect a disillusionment with animal models in immunology. Dr. Krause said that tremendous amounts of information had been derived from animal models, but that difficulties in translating this information into an effective understanding of autoimmune diseases of man have been encountered. Mr. Pauker added that the immunologists at Pasadena did not have a concensus of opinion that the information derived from animal experiments could be directly applied to risk assessment in human beings.

Dr. Gottesman and Dr. Williams reviewed the summary of the Pasadena meeting prepared by the RAC Risk Assessment Working Group (Attachment II), and replied to questions posed by RAC members concerning the Pasadena proceedings. Their recommendations to the RAC are given on page 5 of Attachment II.

V. PROPOSED CHANGES OF THE GUIDELINES TO ALLOW RECOMBINANT DNA EXPERIMENTS WITH CLASS 3 ORGANISMS AND CERTAIN PLANT PATHOGENS

Dr. Gottesman summarized the history of the proposal (tab 881/2). She said NIAID originally requested consideration of whether, and under what conditions, recombinant DNA technology might be used to study CDC Class 3 etiological agents. The issue was presented to the RAC at its December 6-7, 1979 meeting and a working group was appointed to study the issue. A draft proposal was presented to, and subsequently modified by, the RAC at the March 6-7, 1980 meeting. The RAC at that meeting also reviewed the question of whether experiments using plant pathogens should be removed from the prohibitions. It was noted that the mechanism for removing Class 3 agents from the prohibitions could be employed to remove plant pathogens from the prohibitions. The committee requested that language removing plant pathogens and Class 3 agents from the prohibitions be combined and published in the Federal Register for comment.

Dr. Gottesman said the language, published in the April 30, 1980, Federal Register, would remove Class 3 pathogens from prohibition I-D-1 and permit them to be used under P3 containment conditions with *Escherichia coli* K-12 hosts. Containment levels for other experiments would be assigned by the Director, NIH, after review by the RAC.

Dr. Young suggested that the proposed language of Section IV-E-1-b-(2)-(e) be clarified. Dr. Baltimore proposed the following language:

> "IV-E-1-b-(2)-(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens."

Dr. Zaitlin asked if there was any rationale for excluding the clause "beyond that which occurs by natural genetic exchange" from Section IV-E-1-b-(2)-(e) and Section III but including the phrase in Section I-E. Dr. Nightingale suggested that the language of Sections III and IV-E-1-b-(2)-(e) be modified to be consistent with Section I-E, i.e., add after "plant pathogens," the phrase "beyond that which occurs by natural genetic exchange."

Dr. Walters questioned how the proposed amendment would alter Section I-D; he asked if Section I-D-4 would become I-D-3, Section I-D-5 become I-D-4, etc. Dr. Talbot replied that the word "deleted" would be substituted for the current wording in Section I-D-3.

Dr. Nightingale asked how the proposed CDC reclassification effort will affect the NIH Guidelines. Dr. Talbot replied that the 1974 CDC Classification was incorporated into the NIH Guidelines and would remain in Appendix B until the NIH, with RAC advice, promulgates the incorporation of any revised classification into Appendix B.

Dr. Krimsky asked what containment levels are used in working with Class 3 pathogens. Dr. Young replied that most Class 3 pathogens are handled at the P3 equivalent level of containment. Dr. Gottesman moved acceptance of the language as it appeared in the Federal Register with Dr. Baltimore's suggested language in Section IV-E-1-b-(2)-(e) and the clause "beyond that which occurs by natural genetic exchange" added to Sections III and IV-E-1-b-(2)-(e). The proposal was accepted by a vote of fourteen in favor, none opposed, and three abstentions.

VI. CLONING OF FOOT AND MOUTH DISEASE VIRUS - STAGE II

Dr. Baltimore began the discussion of tabs 881/9 and 888 with a brief review of the history of the Plum Island Animal Disease Center (PIADC)-Genentech, Inc., proposal. He noted that at the December 6-7, 1979 meeting the RAC recommended approval, with certain stipulations, of Stage I of the PIADC-Genentech, Inc., proposal to produce a vaccine against Foot and Mouth Disease. Stage I work was the reverse transcribing and cloning of segments of the Foot and Mouth Disease Virus (FMDV) genome at the PIADC facility at Plum Island, New York. He said the RAC deferred approval of Stage II, removal of the clones from Plum Island to the Genentech, Inc., facility in California, until the RAC reviewed the results of Stage I. He said PIADC-Genentech, Inc., had submitted data for review, and were now requesting permission to remove a collection of clones from Plum Island. In keeping with the RAC requirement that the clones to be removed do not contain among them, individually or collectively, the entire FMDV genome, PIADC-Genentech, Inc., proposed removing seventeen clones containing a total of 6,000 nucleotides of the FMDV genome. He said PIADC-Genentech, Inc., proposes to leave at the Plum Island Animal Disease Center three clones containing at least 1,000 nucleotides of the 3' end of the FMDV genome.

Dr. Baltimore said he had two minor reservations concerning the Stage II proposal: (1) whether the segment of the genome to remain at Plum Island is absolutely necessary for infectivity, and (2) the length of the genome. In spite of these minor reservations, Dr. Baltimore said he felt quite confident that the 3' end of the genome to be left on Plum Island is necessary for infectivity. He suggested that if RAC required complete reassurance they might recommend that clone pCT 196 also not be shipped off Plum Island. Dr. Zaitlin said he felt the PIADC-Genentech, Inc., proposal was adequate, as an intact 3' end was necessary for polymerase recognition. Dr. Campbell pointed out that PIADC-Genentech, Inc., had responded to the concerns voiced by RAC members at the December 6-7, 1979 meeting by providing extensive molecular characterization of the material to be shipped. He said he had no difficulty in approving the application as it stands. Dr. Baltimore concurred.

The negative results of the infectivity assays were discussed. Dr. Callis indicated that such tests would be made on all vials containing clones of the FMD genome to be transported off Plum Island. He said the USDA Vectors Committee would evaluate the safety of all materials before granting permission to ship.

Dr. Krimsky noted that some sequencing had been performed at Plum Island. He asked why Stage II could not be performed at Plum Island. Dr. Ross of Genentech, Inc., said that the sequencing could be done on Plum Island, but only at considerable time and expense.

Dr. Baltimore moved approval of the PIADC-Genentech, Inc., proposal as submitted. He said his motion would not require that clone pCT 196 be restricted to Plum Island. By a vote of twelve in favor, two opposed, and four abstentions, the RAC approved the PIADC-Genentech, Inc., proposal to ship the 17 requested clones from the Plum Island Animal Disease Center to the Genentech, Inc., facilities in California.

Dr. Talbot said that at the December 6-7, 1979 meeting the RAC recommended P1 + EK1 containment conditions for experiments performed at the Genentech, Inc., facilities with the clones to be sent from PIADC. He said Dr. Fredrickson deferred approval of these containment conditions at that time until data on Stage I and infectivity assays could be evaluated by the RAC. Dr. Campbell moved that Genentech, Inc., be allowed to work with the clones under P1 + EK1 conditions. Dr. Goldstein asked what containment conditions were employed at Plum Island. Dr. Callis responded that containment on Plum Island is between P3 and P4.

Dr. Setlow called the question and the RAC recommended by a vote of eleven in favor, one opposed, and five abstentions that containment levels for work with the clones at the Genentech, Inc., facilities be set at P1 + EK1.

VII. PROPOSAL TO AMEND SECTION III-O OF THE GUIDELINES TO PERMIT USE OF Ff PHAGES

Dr. Campbell related the history of the proposal (tab 865, 866, 867, 868, 881/1) to include Ff phages under Section III-O of the Guidelines. He said that Dr. Fredrickson, when the January 1980 Guidelines were promulgated, raised the question of whether filamentous F-specific phages should be included under Section III-O. As Section III-O precludes the use of conjugation proficient Escherichia coli K-12, this section could only cover Ff phages grown on hosts which are carrying a transfer-defective plasmid. He said the Phage Working Group met by conference call and, after evaluating the issues, agreed unanimously that Ff phages be included under Section III-O. He then moved approval of the language published in the Federal Register of April 30, 1980.

Dr. Goldstein requested further discussion of the ecology and host-range of the filamentous phages. Dr. Gottesman said that during the conference call, two points were made: (1) the Ff host-range is not as extended as some thought, and (2) the filamentous phages do not stably maintain information cloned into the vector. Dr. Campbell pointed out that these particular male-specific phages are rarely isolated in nature. He said the RNA phages appear to be more abundant, while the DNA phages are rare.

Dr. Setlow called the question. The RAC recommended acceptance, by a vote of fourteen in favor and none opposed, of the language in the Federal Register including Ff phages under Section III-O.

VIII. PROPOSED HV2 BACILLUS SUBTILIS HOST-VECTOR SYSTEMS

Dr. Gottesman introduced the request (tab 875, 881/11, 899) from Dr. William F. Burke of Arizona State University, for HV2 certification of Bacillus subtilis strain ASB298. She said that Bacillus subtilis strain ASB298 contains a variety of mutations. Two sets of mutations block sporulation. A deletion mutation leads to cell lysis under certain growth conditions. Another mutation prevents cell growth in the absence of the rare amino acid, D-alanine. Another mutation confers streptomycin-dependence.

Dr. Gottesman said that an ad hoc group discussed this request by conference call. She said that overall the group was satisfied with the data presented by Dr. Burke. She said the only issue raised concerning a Bacillus subtilis HV2 system was the question of transfer of genetic information from this strain to other Bacillus by transformation. She noted that no mutation Dr. Burke has introduced into this strain blocks transfer out of genetic information through transformation; the only constraint in this Bacillus subtilis system is that ASB298 may not grow very well. Dr. Gottesman added that in Bacillus subtilis, plasmid vectors do not transfer by conjugation. She noted that recombinant material could, however, be transferred by phage vectors and suggested that a Bacillus subtilis HV2 be restricted to plasmid vectors.

Dr. Young said that competent Bacillus subtilis cells can excrete DNA. He felt that any Bacillus subtilis strain excreting DNA is analogous to a conjugation proficient Escherichia coli. Dr. Gottesman said the ad hoc group had discussed the possibility of requesting that Dr. Burke evaluate transformation frequencies in a "flower pot" protocol.

Dr. Novick asked if asporogenic *Bacillus subtilis* strains will persist in soil. Dr. Young replied that asporogenic *Bacillus subtilis* die more rapidly than sporogenic *Bacillus subtilis* but persist for a finite period.

Dr. Gottesman moved to defer consideration of this proposal until additional data are available concerning transfer of DNA by transformation out of ASB298. The RAC approved Dr. Gottesman's motion by a vote of eleven in favor, two opposed, and one abstention.

IX. PROPOSAL TO AMEND SECTION III-B-3 OF THE GUIDELINES

Dr. Campbell introduced this proposal (tab 870, 881/6). He said that during discussions of hosts other than *Escherichia coli* at previous RAC meetings, the recommendation was made to permit cloning, in certain circumstances, in nonpathogenic bacterial hosts other than approved HV or EK hosts. After some discussion, a general provision was put into the Guidelines (Section III-B-3) to permit cloning of prokaryotic DNA in a non-HV certified prokaryote under P3 containment conditions provided neither the DNA cloned nor the host is pathogenic for man, animal, or plants. He said the P3 containment level was not meant to imply that all of these experiments actually require P3 containment. In order to clarify this point, he suggested amended language which parallels the Class 3 pathogen language evaluated earlier in the day. He said the proposed language would introduce one substantive change in Section III-B-3; under the amended language requests to lower containment levels for specific experiments in this class to less than P3 would still require review by the RAC but would no longer require *Federal Register* notice prior to RAC review. He moved approval of the proposal as published in the April 30, 1980, *Federal Register*.

Dr. Krimsky asked Dr. Campbell if two nonpathogenic bacterial species might combine to produce a pathogen. Dr. Campbell said he did not believe this would occur but said he could not predict a chance event. Dr. Gottesman said she thought the review by RAC afforded by the proposed language would permit RAC to consider such possibilities individually. The RAC then recommended approval of the proposed amendment of Section III-B-3 by a vote of thirteen in favor, none opposed, and four abstentions.

X. PROPOSAL TO DELETE LANGUAGE REQUIRING USE OF CaMV MUTANTS LACKING THE APHID TRANSMISSION FACTOR FROM SECTION III-C-3 OF THE GUIDELINES; REQUEST FOR PERMISSION TO TRANSFER DNA FRAGMENTS FROM APHID TRANSMISSIBLE TO APHID NONTRANSMISSIBLE CaMV

Dr. Brill introduced the request (tab 869, 881/7) of Dr. Robert Shepherd of the University of California at Davis. He said Dr. Shepherd requested deletion, from Section III-C-3 of the Guidelines, of language restricting the use of Cauliflower Mosaic Virus (CaMV) as vectors to mutants lacking the aphid transmission factor. Dr. Brill said Dr. Shepherd argues that no other DNA plant viruses are similarly restricted in the Guidelines. Dr. Brill noted that CaMV does not replicate in the aphid and is lost from the aphid in a few hours. Dr. Gottesman asked if the use of non-transmissible CaMV vectors provides an extra measure of containment. Dr. Zaitlin replied that aphid transmissible CaMV strains are easily contained mechanically. He said the virus has a limited host range, and a highly specialized interaction exists between aphid and plant. He said that the use of nontransmissible CaMV strains may provide some additional containment but felt that risk of disseminating recombinant material by this vector was very low.

Dr. Setlow proposed that the two questions Dr. Shepherd posed be voted on separately. Accordingly Dr. Brill moved approval of Dr. Shepherd's request to delete language requiring use of aphid non-transmissible CaMV strains from Section III-C-3. The RAC accepted this motion by a vote of ten in favor, none opposed, and four abstentions.

Dr. Brill then moved that the RAC approve Dr. Shepherd's request to permit introduction of DNA fragments from the genome of aphid transmissible CaMV to the genome of aphid non-transmissible strains under P1 conditions. The RAC accepted this motion by a vote of eleven in favor, none opposed, and three abstentions.

XI. PROPOSED REVISION OF SECTION III-C-1-e OF THE GUIDELINES

Dr. Berns reviewed the history of the proposal (tab 881/12, 900) to revise Section III-C-1-e of the Guidelines. He said that a proposal dealing with the use of animal virus genomes as vectors was presented to the RAC at the March 6-7, 1980 meeting. At that meeting, part I of the proposal was modified and recommended by the RAC, and subsequently approved by the Director, NIH, changing Section III-C-1-e, III-C-1-e-(1), and III-C-1-e-(1)-(a) of the Guidelines.

Dr. Berns said that consideration of parts II and III of the proposal was deferred until the June 5-6, 1980 meeting to permit a working group to examine the issues in greater detail. The working group met in Miami Beach, Florida, in May 1980 during the meeting of the American Society for Microbiology. Dr. Berns said the approval given to part I of the proposal was reevaluated. The concensus of the working group was that P1 containment conditions are acceptable if the investigator has demonstrated the absence of helper virus.

Dr. Berns said the working group then examined the situation wherein defective viral genomes are rescued by helper virus. He noted that the original proposal suggested P2 containment levels for rescue experiments if wild-type strains of the helper virus are not able to grow in human cells. Containment for rescue experiments would be set at P3, if wild-type strains of the helper virus are able to grow in human cells. He stated that the rationale for setting these levels was that a virus capable of infecting humans could disseminate from the laboratory by means of an infected investigator. He said he perceived no support for this notion among the working group in Miami Beach. The working group suggested that the containment level to be used in rescue experiments be set according to the CDC classification of the helper virus; helper rescue with a CDC Class 1 or 2 agent would be carried out under P2 conditions while helper rescue with a CDC Class 3 agent would be carried out under P3 conditions.

Dr. Goldstein asked if the working group had addressed the question of potential changes in host range. Dr. Berns replied that the experience of working group participants suggested that a number of viruses recombine with host genetic material during infection but that this recombination produces neither enhanced virulence, pathogenicity, nor host range.

Dr. Walters noted that the proposal as now formulated had not appeared in the Federal Register, and that it should be published for comment before consideration at the September meeting.

XII. PROPOSAL TO AMEND PORTIONS OF SECTION III-A-2-a OF THE GUIDELINES

Dr. Nightingale outlined the proposal (tab 881/3) to amend portions of Section III-A-2-a. In the January 1980 Guidelines, the term HV1CV had been substituted for the term EK1CV, and does not seem applicable to the host-vector systems currently included in Section III-A-2-a. An ad hoc working group had been appointed to examine this question. The working group had proposed modifying the language of Section III-A-2-a by substituting HV2 containment for HV1CV. The RAC at the last meeting had not accepted the proposed substitution of HV2 for HV1CV; the majority of members thought HV2 containment too stringent. A substitute proposal to amend HV1CV to HV1 was therefore published in the Federal Register for consideration at this meeting.

Dr. Brill moved acceptance of the amendment as it appeared in the April 30, 1980, Federal Register. Dr. Gottesman agreed that the HV1CV level does not make sense. She noted that this motion would lower containment for some cloning of animal viruses in Bacillus subtilis and Neurospora crassa.

Dr. Goldstein moved to table discussion of Dr. Brill's motion. He said the proposed change is a major relaxation in containment of recombinant DNA from eukaryotic viruses. He suggested that a subcommittee be appointed to review available data. The vote was called on Dr. Goldstein's motion. The RAC defeated the motion to table by a vote of two in favor, nine opposed, and three abstentions.

Dr. Setlow called the question on Dr. Brill's motion. The RAC recommended by a vote of nine in favor, and five opposed, that the language of Section III-A-2-a be amended to substitute HV1 containment for HV1CV containment, as specified in the Federal Register of April 30, 1980.

XIII. PROPOSAL TO AMEND SECTION III-C-4 OF THE GUIDELINES

Dr. Zaitlin introduced his proposal (tab 881/8) to amend Section III-C-4 of the Guidelines. He stated that this proposal is an attempt to rectify an oversight in the definition of a P1 greenhouse or growth cabinet when agents other than viruses are used as vectors. He said the definition of a P1 greenhouse or growth cabinet currently in the Guidelines, established for use of plant viruses in recombinant technology, appears in Section III-C-3. As some plant viruses are transmitted by aphids, positive air pressure is required to prevent entry of insects into the P1 greenhouse or growth chamber. In Section III-C-4 of the Guidelines discussing the use of vectors other than viruses, in cases where airborne propagules (spores, mycelia, etc.) could be generated, positive air pressure would actually encourage dissemination of the recombinant molecules. He moved that the following language be added at the end of Section III-C-4:

> "and (iii) negative air pressure shall be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules."

The RAC accepted the proposed language by a vote of fourteen in favor, and none opposed.

XIV. LOWERING OF CONTAINMENT LEVELS FOR CHARACTERIZED CLONES OR PURIFIED DNAs BY IBCs

Dr. Gottesman presented a brief review of the proposal (tab 881/13) to permit local IBCs to lower containmment levels on characterized clones or purified DNAs. She said the issue is whether HV host-vector systems should be treated in the manner EK host-vector systems were treated under the 1978 Guidelines, i.e., the containment of purified DNA or characterized clones shown to be free of harmful genes could be lowered one level in either physical or biological containment by the local IBC without the need for NIH approval. Any further lowering, or lowering below P1 + HV1 would have to be approved by NIH. Under the 1980 Guidelines, such lowering of containment levels for HV systems requires prior IBC and NIH approval.

Dr. Gottesman moved acceptance of the proposal as it appeared in the Federal Register. The RAC unanimously accepted this proposal by a vote of fifteen in favor, and none opposed.

XV. PROPOSED FOOTNOTE REGARDING VARIOLA AND WHITEPOX VIRUSES

Dr. Maas introduced the proposal (tab 881/15) to footnote any mention in the Guidelines of alastrim, smallpox, and whitepox viruses. He said the purpose of the footnote was to call attention to World Health Organization policy regarding these viruses and worldwide efforts at eradication.

Dr. Goldstein asked how smallpox is currently classified by the CDC. Dr. Berns replied that the 1974 CDC classification, compiled before smallpox was eradicated, lists smallpox as Class 3 when used *in vitro* and Class 4 when used *in vivo*. Work with alastrim, smallpox, and whitepox is now restricted by the World Health Organization to a few research and storage centers scattered throughout the world. Dr. Milewski said that the proposed language had been composed by the CDC.

Dr. Maas moved acceptance of the language as it appeared in the *Federal Register*. The RAC accepted this recommendation by a vote of seventeen in favor, and none opposed.

XVI. PROTOCOLS REQUIRING ASSIGNMENT OF CONTAINMENT LEVELS

A. Request for Permission to Transform *Saccharomycopsis lipolytica* with *Escherichia coli/Saccharomyces cerevisiae* Hybrid Plasmids

Dr. Broadbent introduced the request (tab 874, 881/5) of Dr. David Ogrydziak of University of California at Davis for permission to transform *Saccharomycopsis lipolytica* with *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids. Dr. Broadbent said *Saccharomycopsis lipolytica* is non-pathogenic and does not colonize animals. In the absence of pathogenicity or toxin production, Dr. Broadbent recommended P1 containment for the proposed experiments. Dr. Young concurred. He said *Saccharomycopsis lipolytica*, a soil organism, has been used industrially for fermentation and the production of citric acid.

Dr. Campbell asked Dr. Broadbent to clarify whether he wished to recommend P1 containment for Dr. Ogrydziak's proposed experiments or whether he was recommending *Saccharomycopsis* as a HV1 host-vector system. Dr. Broadbent replied that he wished to recommend P1 containment for the specified experiments. He moved acceptance of P1 containment conditions. The RAC accepted the motion by a vote of fifteen in favor, none opposed and one abstention.

B. Request for Use of Conjugative Plasmids and Transducing Phages in an *Escherichia coli* K-12 host

1. Dr. Campbell introduced the proposal (tab 879, 881/19) of Dr. A. I. Bukhari of the Cold Spring Harbor Laboratory. He said Dr. Bukhari wishes to employ conjugative plasmids or transducing

phages in recombinant DNA experiments with E. coli when a small fragment of Adenovirus 2 DNA has been used as linker DNA. He said Dr. Bukhari argues that this experiment is essentially self-cloning and said he tended to agree. Dr. Campbell moved approval of Dr. Bukhari's proposed experiments.

Dr. Goldstein asked which Adenovirus 2 DNA fragment was to be used as the linker. Dr. Baltimore replied that the fragment comes from a region far from the transforming region.

The question was called and the RAC recommended approval of Dr. Bukhari's request by a vote of fourteen in favor, none opposed, and one abstention.

2. Dr. Gottesman initiated discussion of a second proposal (tab 893) submitted by Dr. Bukhari. It was noted that this second proposal had been received after the publication deadline and had not appeared in the Federal Register. Therefore, no action could be taken at the June 6-7, 1980 meeting.

   Dr. Gottesman said Dr. Bukhari proposed to amend the definition of EK1 to permit transient use of conjugation-proficient plasmids or generalized transducing phages; i.e., the host-component could contain these vectors but only if they are used as vehicles to transfer recombinant DNA molecules from one strain to another and all the intermediate strains and transducing lysates are destroyed. She said her general inclination would be to reject Dr. Bukhari's proposal and to continue to review specific requests on a case-by-case basis. Others agreed.

   Dr. Setlow suggested that Dr. Campbell discuss this request and the sentiment of the committee with Dr. Bukhari.

C. Request for Exception to a Prohibition to Clone DNA from a CDC Class 3 Agent

Dr. Maas introduced the request (tab 880, 881/20, 890) of Dr. Sebastiao Baeta Henriques of the Instituto de Ciencias Biologicas, Belo Horizonte, Brazil. Dr. Maas said Dr. Henriques requests an exception to a prohibition in order to clone, in Escherichia coli K-12, DNA from Shistosoma mansonii, a CDC Class 3 etiological agent. He said Dr. Henriques hopes to develop a vaccine against shistosomiasis through the use of recombinant DNA techniques. Dr. Maas said Dr. Henriques' proposal had been discussed briefly at the March 6-7, 1980 meeting but had not been published in the January 31, 1980, Federal Register and could not be acted on in March.

Dr. Young cited the complicated life cycle of the organism and said he could not envision any biohazard developing from cloning Shistosoma mansonii DNA in Escherichia coli K-12. He recommended P1 containment. Several committee members concurred.

Mr. Thornton said that in recommending this proposal to the Director, NIH, the RAC would establish a precedent of acting on proposals from foreign countries where it has no jurisdiction. He wished to avoid such a precedent and proposed that a personal letter be sent to Dr. Henriques from one or more scientists to describe the discussion and the sentiments of the RAC. Several committee members supported this point of view, and Dr. Setlow agreed to inform Dr. Henriques unofficially of the deliberations of the RAC concerning his proposal.

Later in the meeting, Dr. Novick stated that he was dissatisfied with this approach and requested further discussion of the issues. He felt the RAC should treat the proposal as if it had originated in the United States and take an official position by a vote. He so moved. Mr. Thornton opposed this position. He moved to table Dr. Novick's motion. He said a vote to table would be an endorsement of the previously agreed upon procedure. By a vote of ten in favor, one opposed, and six abstentions, the RAC tabled Dr. Novicks' motion.

D. Request for Consideration of Appropriate Containment Levels for the Return of Mucor racemosus Genes Cloned in Saccharomyces cerevisiae to Mucor racemosus

Dr. Maas introduced the request (tab 897, 881/16) of Dr. Paul S. Sypherd of the University of California at Irvine. Dr. Sypherd requested consideration of containment levels appropriate to the return of Mucor racemosus DNA cloned in Saccharomyces cerevisiae host-vector systems to Mucor racemosus. In addition, Dr. Sypherd requested permission to transform Mucor racemosus with Saccharomyces cerevisiae vectors with or without cloned Saccharomyces cerevisiae sequences. Dr. Young said he had no reservation about approving use of this organism at appropriate containment. He expressed concern on the pathogenic nature of certain Mucor species, which can cause Mucormycoses in compromised individuals. He suggested that P2 containment conditions be permitted, but felt the investigator should be alerted to the possible hazards of handling Mucor.

Dr. Gottesman asked if Mucor racemosus was characterized enough to permit evaluation of the effect of introducing fragments of the Saccharomyces cerevisiae genome into Mucor racemosus. Dr. Berns asked how Mucor racemosus was classified. Dr. Nightingale replied that all Mucor species were classified in the proposed revised CDC listing as Class 2 etiological agents. Dr. Berns pointed out that Mucor is not highly pathogenic; he noted that many microorganisms can become pathogens in compromised individuals. Dr. Maas moved acceptance of the proposed experiments at P2 containment. The RAC recommended approval of Dr. Sypherd's proposal by a vote of fourteen in favor, none opposed, and four abstentions.

XVII. PROPOSED FUTURE MAJOR ACTIONS

A. Proposal to Introduce *Arabidopsis* DNA Ligated into an *Escherichia coli*/Ti Hybrid Plasmid into *Arabidopsis*

Dr. Gottesman said that Dr. Donald Merlo of the University of Missouri-Columbia had requested (tab 892) advice from the RAC on the containment appropriate to the following protocol: (1) clone the thiamine (thi) genes from *E. coli* or *Agrobacterium tumefaciens* in a thi$^-$ *E. coli* K-12; (2) clone the origin of replication, and possibly the bacteria-plant transfer genes of the Ti plasmid of *Agrobacterium tumefaciens* in *E. coli* K-12; (3) clone *Arabidopsis* DNA fragments in the pBR325/Ti plasmid (PL2) in step two in *E. coli* K-12 or χ1776; (4) isolate the thiamine gene from the pBR325/thi plasmid (PL1) in step one, and insert them by restriction and ligation into the *Arabidopsis* DNA sequences in the pBR325/Ti/*Arabidopsis* plasmid (PL3) from step three; (5) the reisolated PL4 plasmid would be transformed into *Agrobacterium tumefaciens*; and (6) infect thiamine mutants of *Arabidopsis* with *Agrobacterium tumefaciens* containing PL4.

Dr. Zaitlin said the first four steps are covered by Section III-O of the Guidelines. He noted that *Arabidopsis* can be grown in a test tube and can be easily contained. It was agreed that steps 5 and 6 were covered by Appendix E and that containment should be P3. Dr. Gottesman suggested that as the *Escherichia coli* and *Agrobacterium tumefaciens* DNA fragments are well characterized, and *Arabidopsis* DNA will be returned to *Arabidopsis*, containment for these particular experiments could be P2. She moved approval of steps 5 and 6 at P2 containment. A vote of eleven in favor, none opposed, and five abstentions supported Dr. Gottesman's motion. (Executive Secretary's Note: Dr. Merlo's request will be reconsidered at the next RAC meeting following publication in the *Federal Register*.)

B. Review of Section I-D of the Guidelines

Dr. Nightingale said that an *ad hoc* group had been formed following the March 6-7, 1980 meeting to determine whether Section I-D of the Guidelines required revision. Their report is tab 894.

Dr. Nightingale noted that Section I-D-1, prohibiting formation of recombinant DNAs derived from CDC Class 3, 4 or 5 agents, had been recommended for modification at this meeting so as to permit experiments with Class 3 agents while still prohibiting use of Class 4 and

5 agents. She noted that a proposed revision of the CDC classification of etiological agents will soon be issued. Dr. Berns said he favored waiting for the CDC revised classification to appear before considering further modifications in Section I-D-1. He felt that the revised classification, because of the number of individuals participating in the review, would be better than any RAC could construct. He thought the RAC review process afforded by the Guidelines was adequate until the revised CDC classification became available. Dr. Goldstein questioned Dr. Dowdle of CDC on the status of the CDC revision. Dr. Dowdle said reviewers' comments had been received and that substantial modifications of format, as well as classification of some organisms would be incorporated into a revised document, and that a draft will be circulated to the RAC.

Dr. Gottesman said she was inclined to maintain the status quo. She said that there had been no requests for permission to do recombinant DNA experiments employing CDC Class 4 or 5 organisms. Dr. Setlow said she sensed a concensus for maintaining the status quo and suggested Dr. Nightingale begin discussion of the next item.

Dr. Nightingale noted that Section I-D-2 prohibits "deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (2A) (e.g., botulinum or diphteria toxins; venoms from insects, snakes, etc.)" She said an ad hoc group chaired by Dr. Maas was attempting to define what is a potent toxin. Dr. Maas said he had composed a table (tab 895) outlining the lethal dose of various bacterial toxins. He said the table has limitations in that (1) the amount of information available is limited; (2) lethality is measured for experimental animals, and humans may react quite differently; and (3) deleterious effects other than lethality have not been considered. He suggested that perhaps only the cloning of botulinum and tetanus toxins should be prohibited. Cloning of other toxins could be considered on an individual basis. Dr. Campbell expressed concern that human sensitivity to a toxin may differ significantly from that of animal test systems. Dr. Ahmed said that enough uncertainty existed in the data to render consideration of the proposal very difficult. Dr. Young suggested that potent toxins might be dealt with generically by removing them from the prohibitions and setting containment at P4. Dr. Levine said botulinum toxin is in a class by itself; extraordinarily small quantities can kill by the oral route. He said tetanus toxin can also kill in quantities too small to elicit an immune response, but must be administered parenterally.

Dr. Nightingale pointed out that plant toxins, some of which are very potent, are not covered by the prohibitions. She suggested that this type of toxin be reviewed by an appropriate group. Dr. Young said he felt the bacterial toxins should be examined first, toxins from other sources could be evaluated at a later date. Drs. Maas and Young agreed to prepare a proposal for consideration at a future meeting.

Dr. Nightingale noted that Section I-D-3 had been dealt with earlier in the meeting and said that the working group felt the other prohibitions should not be changed at the present time.

C. Request for Certification of a *Bacillus stearothermophilus* Plasmid as the Vector Component of an HV1 *Bacillus subtilis* Host-Vector System

Dr. Young introduced the request (tab 906) of Dr. David B. Wilson of Cornell University for certification of a *Bacillus stearothermophilus* derived plasmid, pAB124, as the vector component of an HV1 *Bacillus subtilis* host-vector system. He said *Bacillus stearothermophilus* is a non-pathogenic thermophile found in soil near hot springs, on compost, etc. He said Dr. Wilson argues that the plasmid pAB124 can be transformed into *Bacillus subtilis*, and it should be considered an endogenous plasmid. Dr. Young recommended approval.

Dr. Gartland noted that this plasmid could be used with the only HV1 certified *Bacillus subtilis* strain, RUB331. He noted that the proposal had not been published in the *Federal Register*. The Committee agreed that this proposal should be published in the *Federal Register* and discussed at the September 1980 meeting.

XVIII. PROPOSALS TO AMEND SECTION I-D-6 OF THE GUIDELINES

Dr. Walters introduced a series of proposals (tab 877, 881/17/A, 881/17/B, 881/18/A, 881/18/B, 881/18/C) from Dr. Irving Johnson of Eli Lilly and Company, to amend the Guidelines. He noted Dr. Johnson, in 881/17/A, proposed to amend Section I-D-6 of the Guidelines by deleting the sentence:

> "We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale."

Dr. Walters recommended approval of the proposal. He said the generalization about the probability of escape is not accurate, as for example, if small-scale work were done in open containers and large-scale work were done in a closed system. Mr. Thornton concurred noting that the language is a prefatory statement and its deletion would not change the containment standards of the Guidelines. Drs. McKinney and Campbell said that any single escape may result in more organisms being released, but the probability of a single breach occurring need not increase with increasing volume. Dr. Baltimore noted that containment is a function of the machinery used as well as of the volume. Dr. Walters moved to delete the sentence. The RAC passed this motion by a vote of fourteen in favor, one opposed, and one abstention.

Mr. Thornton initiated discussion of Dr. Johnson's proposal (881/17/B) to amend Section I-D-6, by adding the sentence:

> "Those experiments utilizing an E. coli K-12 host-vector system and meeting the containment requirements of Section III-O are excluded from these prohibitions."

Dr. Walters said he found the statement to be too broad. It could be interpreted as removing all experiments using E. coli K-12 as the host from all the prohibitions. Dr. Walters assumed it was Dr. Johnson's intention only to remove prohibition I-D-6 for these experiments. Dr. Walters recommended denying the proposal and so moved. Dr. Johnson said he had intended the proposal only to deal with prohibition I-D-6. Dr. Campbell said that Section III-O was based on consideration of probability of effect of small-scale manipulations. He said that logic does not automatically apply to large-scale manipulations. He opposed the proposal.

The RAC denied 881/17/B by a vote of seventeen in favor, none opposed, and one abstention.

XIX. PROPOSALS TO AMEND SECTION IV-E-2 OF THE GUIDELINES

A. Proposal to Amend Section IV-E-1-b-(3)-(d)

Mr. Thornton initiated discussion of Dr. Johnson's proposal (tab 877, 881/18/A) to amend Section IV-E-1-b-(3)-(d) by the addition of the following paragraph:

> "Further volume increases at the approved containment level, e.g., for industrial development and production purposes, of previously approved (by the Director of NIH) large-scale experiments may be subsequently approved by the local IBC in accordance with Section IV-D-3-a. The IBC will notify ORDA of such volume increases."

Dr. Walters said he preferred to maintain the principle of external review or validation of equipment, although this review need not be conducted by the RAC. It was pointed out that this proposal might more properly be considered with Dr. Gottesman's proposal concerning RAC's role in reviewing large-scale applications. A motion to table the proposal passed by a vote of eighteen in favor, none opposed.

B. Proposal to Add a New Subsection to Section IV-E-2-b

The committee considered Dr. Johnson's proposal (tab 877, 881/18/C) to add a new subsection IV-E-2-b to the Guidelines to read as follows:

> "IV-E-2-b. A permanent subcommittee of the RAC shall be responsible for advising the Director of NIH, on the actions listed in Section IV-E-1-b-(3)-(d) pertaining to large-scale applications. Submissions that are in compliance with the Guidelines may be recommended by the subcommittee to the Director of NIH for approval. The subcommittee shall also be authorized to consider preliminary plans for large-scale operations and to recommend approval contingent upon completion of the large-scale facility according to these plans. The subcommittee will be responsible for expeditiously processing applications."

Mr. Thornton said the proposal would be more properly considered at the September 1980 meeting when the RAC would be voting on the implementation of the principles voted earlier in the meeting (Section II of these minutes). A motion to table the proposal passed by a vote of eighteen in favor, none opposed.

C. Proposal to Amend Section IV-E-2 of the Guidelines

Ms. Cason introduced Dr. Johnson's proposal (tab 877,881/18/B) to amend Section IV-E-2 of the Guidelines, by adding the following sentence:

> "Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

Ms. Cason agreed that it would be desirable to have someone with expertise in fermentation technology appointed as a RAC member, particularly if RAC continues to review the details of physical containment in applications submitted under the program of voluntary compliance. She disagreed, however, with Dr. Johnson's language requiring that the person be a "representative of industry" and proposed that the sentence should read as follows:

> "Members should also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

Dr. Talbot pointed out that if the RAC recommended Dr. Johnson's proposal, this would require an amendment to the RAC Charter, and such an action cannot be approved by the Director, NIH. Rather, the Secretary of the Department of Health and Human Services (HHS) takes final action on recommended modifications to the charters of the HHS the HHS public advisory committees.

Dr. Zaitlin supported Ms. Cason's position. He found the stipulation "representatives of industry" inappropriate. Dr. Campbell moved acceptance of Ms. Cason's proposed language. The RAC accepted Ms. Cason's proposal by a vote of ten in favor, five opposed, and two abstentions.

Dr. Young said that even though he found Ms. Cason's language appropriate, he felt that consideration of this issue should be deferred until the September 1980 meeting, and moved to reconsider the vote. The RAC accepted Dr. Young's motion to reconsider, by a vote of ten in favor, five opposed, and two abstentions.

Dr. Young then moved to defer consideration of the proposal until the September 1980 meeting. Dr. Berns said he opposed the motion to defer; he felt RAC needed this type of expertise. He noted that American Society for Microbiology strongly suggested that an expert knowledgeable in fermentation technology be appointed to RAC. Mr. Thornton said he supported the motion to defer. He felt that no recommendation should be sent to the Secretary, HHS, in advance of the RAC review in September of procedures regarding large-scale applications. By a vote of thirteen in favor, two opposed, and three abstentions, the RAC deferred further discussion of the proposal until September 1980.

XX. PROPOSED PROCEDURES FOR MINOR MODIFICATIONS IN LARGE-SCALE RESEARCH OR PRODUCTION

Dr. Walters introduced the proposal (tab 887) of the Working Group on Large-Scale Procedures to revise review procedures for minor modifications of previously approved large-scale protocols. He said three types of modifications can be envisioned: (1) changes in containment vessels, (2) changes in host-vector systems, and (3) changes in inserted DNA.

Under proposed modification, RAC would not evaluate changes in containment systems; system design and validation data would be reviewed and approved by ORDA or its designee. Dr. Johnson said that his proposal to modify review procedures for large-scale applications was intended to achieve an objective similar to that proposed by the working group. Dr. Baltimore questioned whether changing from a 150-liter fermentor system to a 10,000 gallon fermentor system should be considered a minor modification. Dr. Gottesman moved that discussion of this proposal be deferred until September. The RAC voted in favor of the motion by a vote of eighteen in favor, none opposed.

Dr. Walters then described part two of the proposal. He said criteria previously developed to identify and review minor modifications in host-vector systems used in small-scale manipulations would be applied to large-scale manipulations. Review and approval of minor modifications of host-vector systems used in large-scale manipulations would thus be reviewed by at least one member of a working group with a three-week comment period for all members of the working group.

Dr. Gottesman said she did not believe that the small-scale standards which were written specifically for minor modifications of previously approved HV2 systems (Attachments II-IV to the RAC minutes of May 21-23, 1979) were applicable to all changes in host-vector systems. They include no language discussing changes either in the protein expressed or the level of protein expression. She said that while she favored the concept of expedited review for minor modifications of previously approved applications, new language defining such minor modifications should be constructed, and moved deferral. The RAC voted in favor of the motion by a vote of eighteen in favor, none opposed.

The RAC then discussed part three of the proposal dealing with changes in the inserted DNA. Dr. Berns asked if a harmful product could ever be produced by deleting sequences from a gene producing no harmful product. Dr. Williams suggested that part three of the proposal should also be considered with parts one and two, and moved deferral until September 1980. The RAC voted in favor of the motion by a vote of seventeen in favor, none opposed.

XXI. PROPOSAL TO INCLUDE SACCHAROMYCES CEREVISIAE HOST-VECTOR SYSTEMS UNDER SECTION III-O OF THE GUIDELINES

Dr. Setlow introduced Dr. Gerald Fink of Cornell University, an *ad hoc* consultant. Dr. Broadbent reviewed the history of the proposal (tab 871, 872, 873, 881/4, 891) to include laboratory strains of *Saccharomyces cerevisiae* under Section III-O of the Guidelines. He noted that Dr. Setlow at the March 6-7, 1980, RAC meeting proposed this amendment to the Guidelines. He summarized the arguments supporting inclusion of *Saccharomyces cerevisiae* under Section III-O: (1) *Saccharomyces cerevisiae* is sensitive to autoclaving and disinfecting agents, (2) *Saccharomyces cerevisiae* does not colonize animals, (3) *Saccharomyces cerevisiae* has been consumed by humans in both food and drink for millenia with no ill effects, (4) the laboratory strains of *Saccharomyces cerevisiae* do not compete or survive well in nature, (5) the possibility of transmission of recombinant DNA from laboratory to wild-type strains of *Saccharomyces cerevisiae* is extremely small, and (6) mammalian genes cloned in *Saccharomyces cerevisiae* are not automatically expressed. Dr. Broadbent moved to recommend incorporation of this proposal into the Guidelines. Dr. Williams supported Dr. Broadbent's motion.

Dr. Campbell discussed the sentence of the proposed text of Section III-O which reads:

> "An exception which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K-12 or *Saccharomyces cerevisiae* efficiently express as a protein product, the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism."

He questioned whether the statement would apply to self-cloning experiments involving *Saccharomyces cerevisiae*. He suggested the reference to *Saccharomyces cerevisiae* in this sentence be struck.

Dr. Gottesman asked if there were any product, produced by a recombinant-containing *Saccharomyces cerevisiae* host-vector system, which might have an ecologically unfavorable effect. Dr. Fink said that *Saccharomyces cerevisiae*, unlike *Escherichia coli*, exports very few protein products. He felt it would be difficult to engineer *Saccharomyces cerevisiae* to export polypeptide products. Dr. Novick asked if *Saccharomyces cerevisiae* participates in genetic exchanges with organisms other than the appropriate *Saccharomyces cerevisiae* mating type. Dr. Fink replied that it did not. He added that mating with the mating type was difficult to affect even in the laboratory situation.

Dr. Goldstein asked if *Saccharomyces cerevisiae* can be engineered to express eukaryotic genes. Dr. Baltimore said that while *Saccharomyces cerevisiae* will not recognize and automatically express eukaryotic genes, the system can probably be engineered to do so.

Dr. Walters moved to amend the proposed language by deleting the words "or *Saccharomyces cerevisiae*" from the sentence beginning "An exception however...." The RAC voted in favor of the motion by a vote of fifteen in favor, one opposed, and three abstentions.

Dr. Broadbent's motion to adopt 881/4 with the modifications of the previous motion was accepted by the RAC by a vote of fourteen in favor, two opposed, and two abstentions.

XXII. REQUEST FOR AN EXCEPTION TO A PROHIBITION TO FIELD TEST CORN GENES IN CORN

Dr. Brill introduced the request (tab 889, 881/10) of Dr. Ronald Davis of Stanford Medical Center to field test corn plants (*Zea mays*) which have been transformed by corn DNA or modified corn sequences cloned in *Escherichia coli* or *Saccharomyces cerevisiae* host-vector systems. Dr. Brill noted that this request requires an exception to Section I-D-4 which prohibits deliberate release into the environment of any organism containing recombinant DNA. Dr. Brill said he could not envisage any danger to animals or plants from these experiments. He felt the experiment to be a very important one and moved acceptance of Dr. Davis' proposal. Dr. Zaitlin concurred, although he noted that Dr. Davis had not indicated the method by which the corn will be transformed nor the vector to be used.

Dr. Novick said that agricultural plants do not have the same potential for dissemination as do wild plants. Dr. Scandalios said that corn genetic material can be dispersed through the pollen. Dr. Tolin noted that dispersion can be prevented by detasseling. She said that the shoots or ear can also be bagged, preventing fertilization and seed formation. She added that harvesting the ears before maturity would also prevent dispersal, as only mature seeds can propagate. Dr. Baltimore noted that seed companies must continually deal with the problem of cross-fertilization of seed grain, and appear to control crosspollenization quite effectively. Dr. Broadbent pointed out that the experiments were to be conducted in an area where no substantial amounts of corn are grown.

The RAC recommended approval of this proposal by a vote of eleven in favor, none opposed, and five abstentions.

XXIII. PROPOSAL TO AMEND SECTION III-C-7-b OF THE GUIDELINES

Dr. Brill introduced the proposal (tab 889, 881/14) made by Dr. Setlow to include in the Guidelines a provision to permit plant DNA cloned in Saccharomyces cerevisiae to be subsequently returned to the plant. Dr. Setlow had suggested that the first sentence of Section III-C-7-b be amended to read as follows:

> "III-C-7-b. DNA from any nonprohibited source (Section I-D) which has been cloned and propagated in E. coli or S. cerevisiae under appropriate containment conditions, may be transferred with the E. coli or S. cerevisiae vector used for cloning to any higher plant organisms (Angiosperms or Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A]."

Dr. Baltimore pointed out that Section III-C-7-b already applies to DNA cloned in Escherichia coli K-12 host-vector systems.

Dr. Zaitlin moved acceptance of the proposal. The RAC recommended approval by a vote of twelve in favor, none opposed, and six abstentions.

XXIV. VALEDICTORY STATEMENT OF THE RETIRING CHAIRPERSON

Dr. Setlow delivered to the RAC the following impressions of her tenure as chairperson:

> "About two and one-half years ago, the previous chairman, Hans Stetten talked to me about the job. He said the job itself is awful, but you have one benefit which is that you get to meet some interesting people. I would agree with the second thing, but he did not adequately warn me about the first. For example, I had no idea that RAC deliberations would end up being positively impolite at times. So, it turned out the only previous experience that prepared me for any of this was raising four fractious young.
>
> As all of you know by this time, I have developed in the last years a passionate interest in the elimination of restrictions for most recombinant DNA work. This passionate interest, I think, was shared by my predecessor, Hans Stetten, as you all know because you probably read his beautiful valedictory speech in "Gene." We have come a tremendous way during the last two years. For example, it is a source of tremendous satisfaction to me that recombinant molecules are no longer considered to be highly dangerous entities and that means that we, as a committee, have totally abandoned the idea of requiring all human sex to go on in P4 facilities.
>
> Having attended every meeting of this Committee since the very beginning, I am good and ready to say goodbye, as many of you know, and I want to thank all of you who have worked extraordinarily long hours to do the chores of this Committee.
>
> I want to say a word about how enormously happy I am about who the new chairman is. I found him to be a fantastically wonderful mixture of the following: competence, affability, polite ferocity and humor. All of these attributes will mean that he will be well able to deal with the awful parts of the new job.
>
> In finishing my remarks, I want to draw your attention to the person who I think is the unsung hero of recombinant DNA and he has been, for a number of years, subjected to endless battering from above, below, and sideways. Scientists, distinguished and otherwise, have had the habit of calling him on the telephone and telling him he is a miserable bureaucratic worm. Will you all please remember that Bill Gartland has been endlessly helpful even during the process of gradual destruction of his own job by the RAC. Thus, I want to pay tribute to Bill Gartland and to his extremely competent ORDA staff, Stanley Barban and Elizabeth Milewski, and that perfect paragon of efficiency, Betty Butler, and her cohorts, Becky Connors and Trisha Stein, and I want to thank them all."

XXV. PROCEDURES FOR REVIEW OF REQUESTS TO GO OVER 10 LITERS (Continued)

Dr. Gottesman presented proposed language based on committee motions on the procedures for review of requests to go over 10 liters in volume (Section II of these Minutes). She said she had sought and incorporated comments from Drs. Young, Goldstein and Krimsky. She read the following proposed language:

> "Motion to be published in the Federal Register for final consideration at the September 1980 RAC meeting:
>
> The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA:
>
> 1. RAC will determine if a given recombinant DNA-containing strain is in fact 'fully characterized and free of harmful sequences.' Such a determination shall include specification of a containment level (P-LS).
>
> 2. These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industrial scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC and appropriate regulatory agencies.
>
> While the RAC will focus its deliberation on scientific analysis and specification of containment (P-LS) procedures at the industrial level, we urge:
>
> 1. that enforcement of these containment guidelines be included in any regulatory process;
>
> 2. that inclusion of appropriate medical surveillance programs be seriously considered."

Dr. Gottesman expressed hope that the RAC would agree on language for publication in the Federal Register. She said she had already received a suggestion that the words "rigorously characterized and the absence of harmful sequences established," be substituted for the phrase "...fully characterized and free of harmful sequences."

Dr. Ahmed suggested that the language of the clause "that enforcement of these containment guidelines be included in any regulatory process" be clarified. He said "regulation" implies "enforcement." Dr. Goldstein said the proposed language attempted to ensure that the issue would be dealt with properly by another agency. Dr. Baltimore objected to the proposal as the thinking of the RAC. He said that other Federal agencies have procedures for instituting regulatory processes, and RAC plays no role in these processes. Dr. Baltimore said the NIH Guidelines provide a flexible framework for evaluating potential hazard associated with recombinant DNA technology; he feared Federal regulations would prove to be inflexible. He said that the NIH Guidelines, are appropriate for the NIH and RAC, but inappropriate for regulatory agencies. Dr. Williams said that the first two items of the proposal conformed to the directives of yesterday's sense of the Committee vote. He said the additional language beginning "While the RAC will focus..." was inappropriate; since it seems to be directing the regulatory process of another agency. Dr. Walters concurred. Dr. Krimsky disagreed; he said it was appropriate for the RAC to express the sentiment that some type of ongoing surveillance should exist. Dr. Goldstein supported Dr. Krimsky's position. He said the language was intended to urge the Interagency Committee to deal with the problem of industrial compliance.

Dr. Berns said he agreed with Drs. Williams and Baltimore, but suggested in addition to deleting the text beginning with "While the RAC will focus..." that the last sentence of item two "Adherence to the specified containment conditions is the responsibility of the local IBC and appropriate regulatory agencies" also be struck. Dr. Williams agreed with Dr. Berns; he said it was not reasonable to try to state two dissimilar objectives in one document, viz.: (1) clarification of the RAC role in voluntary compliance and (2) an attempt to catalyze action by other agencies. Dr. Nightingale suggested that a vote be taken separately on each of these issues. In order to separate the two issues, Dr. Gottesman moved to delete the end of the proposal beginning with "While the RAC will focus...." The RAC accepted Dr. Gottesman's motion by a vote of fifteen in favor, three opposed.

Dr. Berns then moved to delete the second sentence of item two "Adherence to ...and appropriate regulatory agencies." Dr. Goldstein opposed this motion. He felt it important to have oversight of physical containment. Dr. Campbell proposed an amendment to Dr. Bern's motion, he suggested that the last four words of item two "...and appropriate regulatory agencies" be deleted rather than the whole sentence. This would leave the sentence as "Adherence to the specified containment conditions is the responsibility of the local IBC." Dr. Berns accepted this amendment. Dr. Goldstein said he preferred to see some surveillance beyond the local IBC. Dr. Baltimore said he supported Dr. Campbell's amendment.

The RAC then accepted Dr. Campbell's motion to delete the last four words of item two by a vote of thirteen in favor, three opposed, and one abstention.

The question was then called on a motion to approve paragraph one (with the editorial modification) and paragraph two as modified above. The RAC accepted the motion by a vote of seventeen in favor, none opposed, and one abstention.

Dr. Goldstein then moved acceptance of the language at the end of the original proposal which read as follows:

> "While the RAC will focus its deliberation on scientific analysis and specification of containment (P-LS) procedures at the industrial level, we urge:
>
> 1. that enforcement of these containment guidelines be included in any regulatory process;
>
> 2. that inclusion of appropriate medical surveillance programs be seriously considered."

Dr. Novick noted that the language singled out industrial applications. Dr. Krimsky said that passage of this motion was important in telling the regulatory agencies that RAC sees an urgency in this area. Dr. Baltimore said he believed NIOSH and OSHA had more important things to do than worrying about the potential, very theoretical hazards of recombinant DNA. Dr. Campbell said if the regulatory agencies move recombinant DNA up on their agenda, something else will be moved back. The RAC voted against the proposed language by a vote of four in favor, eleven opposed, and three abstentions.

The language of the proposal to be published in the Federal Register for consideration at the September 1980 meeting reads as follows:

> "The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. The RAC will determine if a given recombinant DNA-containing strain is rigorously characterized and the absence of harmful sequences established. Such a determination shall include specification of a containment level (P-LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industrial scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC."

XXVI. FUTURE MEETING DATES

The next RAC meeting will be September 25-26, 1980. January 8-9, 1981, was tentatively selected as the meeting date for the following RAC meeting. Mrs. Butler will contact RAC members to arrange meeting dates for the rest of 1981.

XXVII. CLOSED SESSION

Dr. Setlow introduced the four large-scale consultants: Dr. Glenwood Achorn, Higdon, Arkansas; Dr. Elmer Gaden, University of Virginia, Charlottesville; Dr. Robert McKinney, Enviro Control, Rockville, Maryland; and Dr. Emmett Barkley, Director, Division of Safety, NIH. The RAC then went into closed session to consider proposals from commercial concerns for scale-up of recombinant DNA experiments.

XXVIII. ADJOURNMENT

The meeting was adjourned at approximately 4:45 p.m., Friday, June 6, 1980.

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland J.

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

[illegible] 20 1980
Date

Jane K Setlow
Jane K. Setlow, Ph.D.
Chairman
Recombinant DNA Advisory Committee

federal register

Tuesday
July 29, 1980

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724).

EFFECTIVE DATE: July 29, 1980.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several major actions under the NIH Guidelines for Research Involving Recombinant NDA Molecules. These proposed actions were published for comment in the **Federal Register** of April 30, 1980, and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on June 5–6, 1980. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment. I am deferring action on one major action, recommended for approval by the RAC, proposing the field testing of corn plants (*Zea mays*) into which corn recombinant DNA has been added using an *E. coli* or *S. cerevisiae* vector. A final decision on this proposal will be reached after the receipt of additional information.

Part I of this announcement provides background information on the actions.

Part II provides a summary of the major actions.

I. Decisions on Actions Under Guidelines

I–A. Proposal To Include Saccharomyces Cerevisiae Hose-Vector Systems Under Section III–O of the Guidelines

Dr. Jane Setlow of the Brookhaven National Laboratory, at the March 6–7, 1980 Recombinant DNA Advisory Committee (RAC) meeting, proposed that NIH consider classifying experiments involving laboratory strains of *Saccharomyces cerevisiae* as host-vector systems under Section III–O of the Guidelines. Dr. Setlow advanced the following arguments to support this proposal: (1) *S. cerevisiae* is nonpathogenic; (2) *S. cerevisiae* does not implant in the mammalian intestine; (3) the dilute conditions in which *S. cerevisiae* is found in nature are extremely unfavorable for mating; and (4) laboratory strains do not efficiently compete with wild type strains *S. cerevisiae.* She added that *S. cerevisiae* does not have a biological association with man.

The RAC discussed this issue at the March 6–7, 1980 meeting, and agreed that the proposal should be published in the **Federal Register** to permit thirty days of comment. Accordingly, proposed language which would amend two sections of the Guidelines, appeared in the **Federal Register** of April 30, 1980 [45 FR 28905]. This involved a proposed substitution for Section III–O of the Guidelines and a deletion in Appendix D, as follows:

> III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).
>
> Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host-vector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid bacteriophages or non-conjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae,* laboratory strains shall be used.] for these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 or *S. cerevisiae* efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.
>
> Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).
>
> For those not on the Appendix A list but which exchange genetic information (35) with *E. coli,* experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.
>
> The language "*HV1-Unmodified laboratory strains of *Saccharomyces cerevisiae*" would be deleted from Appendix D.

During the thirty-day comment period, no comments were received.

At the June 5–6, 1980 meeting, the RAC once again discussed this issue. It was noted that *S. cerevisiae* does not colonize animals, large amounts of yeast are regularly consumed by humans with no ill effects, *S. cerevisiae* does not appear to express mammalian genes, and the probability that genetic material will be transferred from or otherwise disseminated by laboratory strains of *S. cerevisiae* is very low.

The RAC suggested that the language of the sentence of the proposed amended Section III–O which begins, "an exception, however * * *" might lead to confusion over the status of cloning *S. cerevisiae* DNA in *S. cerevisiae.* A motion that the phrase "or *S. cerevisiae*" be deleted from this sentence passed by a vote of fifteen in favor, one opposed, and three abstentions. The amended sentence would read:

> * * *An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

The proposal to include *S. cerevisiae* host-vector systems under Section III–O of the Guidelines as published in the **Federal Register** of April 30, 1980 with the amendment noted above, was accepted by the RAC by a vote of fourteen in favor, two opposed, and two abstentions.

I accept this recommendation.

In addition to the deletion of reference to HV1 *S. cerevisiae* in Appendix D of the Guidelines, reference to HV2 *S. cerevisiae* has also been deleted from Appendix D. Added to Appendix D, however, is reference to RUB 331 which has been certified as the host component of *B. subtilis* HV1 systems. Added to Appendix E is a description of certified EK2 and HV2 *E. coli* and *S. cerevisiae* systems.

I-B. Proposal To Amend Section III-O of the Guidelines To Permit Use of Ff Phages

In January 1980 (45 FR 6724) the National Institutes of Health (NIH) promulgated revised Guidelines. The revised Guidelines included a Section, III-O, "Classification of Experiments Using the *E. coli* K-12 Host-Vector Systems," which reads, in part, as follows:

> * * * experiments using *E. coli* K-12 shall use Pl physical containment and * * * an EK1 host-vector system [i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid bacteriophages or nonconjugative plasmids shall be used as vectors].* * *

No reference to the use of Ff bacteriophages (filamentous single-strand male-specific bacteriophages such as M13 and fd) was made in Section III-O. Consequently, when the revised Guidelines were promulgated in January 1980, investigators were informed that Ff bacteriophages might continue to be used as a component of EK1 systems at the containment levels specified by the 1978 Guidelines.

In December 1979, the RAC was asked to consider how *E. coli* K-12 host-vector systems employing Ff phages should be treated under the 1980 Guidelines. In response to this NIH request, the Host-Phage Subcommittee drafted a recommendation which was presented to the full RAC at the March 6-7, 1980 meeting. The RAC agreed that the proposed amended language of Section III-O should be published in the **Federal Register** for thirty days of comment. Accordingly, the following language appeared in the **Federal Register** of April 30, 1980 [45 FR 28904]:

> * * * experiments using *E. coli* K-12 shall use Pl physical containment and, * * * an EK1 host-vector system [i.e., (a) the host shall not contain conjugation-proficient plasmids or generalized transducing phages and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids shall be used as vectors].* * *

No comments on this proposal were received during the thirty-day comment period.

At the June 5-6, 1980 meeting, the RAC once again considered this issue. The safety of Ff phages was discussed. By a vote of fourteen in favor, none opposed, the RAC recommended that the Host-Phage Subcommittee proposal to include Ff phages under Section III-O be accepted.

I accept this recommendation.

I-C. Proposed Changes To Allow Recombinant DNA Experiments With Class 3 Organisms and Certain Plant Pathogens

In December 1979, the National Institute of Allergy and Infectious Diseases (NIAID) requested that the NIH consider whether, and under what conditions, recombinant DNA technology might be used to study CDC Class 3 etiological agents. Currently Section I-D-1 of the Guidelines prohibits:

> Formation of recombinant DNAs derived from the pathogenic organisms classified [1] as Class 3, 4 or 5 [2] or the cells known [2A] to be infected with such agents, regardless of the host-vector system used.

This issue was presented to the RAC at its December 6-7, 1979 meeting and a working group was appointed to study the issue. A draft proposal which would revise the Guidelines so as to remove Class 3 agents from Section I-D-1 of the Guidelines was subsequently presented to the full RAC at its March 6-7, 1980 meeting.

At the March 6-7, 1980 meeting, a request from Dr. Clarence Kado of the University of California, Davis, to delete Section I-D-3 of the Guidelines was also presented to the RAC. Section I-D-3 prohibits:

> Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange. [2A]

During the discussion of this issue it was suggested that the mechanism used to remove CDC Class 3 agents from Section I-D-1 of the Guidelines might be appropriate for removing recombinant DNA experiments involving certain plant pathogens from Section I-D-3. Accordingly, the proposals have been combined.

The proposed changes would remove recombinant DNA experiments with CDC Class 3 organisms and certain recombinant DNA experiments with plant pathogens from the prohibited classes of experiments. The proposal would permit experiments with CDC Class 3 organisms to be conducted at P3 containment in *E. coli* K-12 EK1 host-vector systems. The containment levels for other recombinant DNA experiments would be assigned by the Director, NIH after review by the RAC.

It is felt that the proposal would reduce administrative procedures, thereby increasing flexibility and encouraging the use of recombinant techniques to elucidate the pathogenic process. It was reasoned that P3 containment, which is considered adequate for work with the pathogenic organisms themselves, should be adequate, or more than adequate, for work with *E. coli* K-12 containing recombinant DNA from these organisms.

The proposed amendments to the Guidelines appeared in the **Federal Register** of April 30, 1980 [45 FR 28904] as follows:

> A. Section I-D-1 is to be amended to read:
>
> I-D-1. Formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known [2A] to be infected with such agents, regardless of the host-vector system used.
>
> B. Section I-D-3 is to be deleted.
>
> C. Section I-E is to be amended to read:
>
> I-E. Exemptions. It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I-D-1 to I-D-5 as being prohibited.
>
> In addition, any recombinant DNA molecules involving DNA from Class 3 organisms (1) or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I-E-5.
>
> D. Section III. After the last sentence, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:
>
> Experiments involving recombinant DNA from Class 3 organisms (1) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K-12 EK1 hosts (see Section III-O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen will be determined by NIH (See Section IV-E-1-b-2-e).
>
> E. Section III-O. After the third paragraph, 'Some experiments * * * are exempt from the Guidelines (see Section I-E),' the following new paragraph is to be added:
>
> Experiments using *E. coli* K-12 EK1 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See section IV-3-1-b-2-e).
>
> F. A new Section IV-E-1-b-2-e is to be added as follows:
>
> IV-E-1-b-2-e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and for experiments which increase the host-range and virulence of plant pathogens.
>
> G. Section V, footnote 2, is to be amended to read:
>
> 2. For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.
>
> H. Section V, footnote 36. The second sentence is to be amended to read:
>
> (As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

During the thirty-day comment period, no comments were received. The RAC discussed this issue at the June 5–6, 1980 meeting. It was suggested that the wording "beyond that which occurs by natural genetic exchange" in Section I–E should be added to Section III and Section IV–E–1–b–2–e following the clause "* * * which increases the virulence and host range of a plant pathogen." In addition, it was suggested that the language of Section IV–E–1–b–2–e shoud be clarified by addition of the phrase "assigning containment levels." A modified Section IV–E–1–b–2–e would read:

> IV–E–1–b–2–e. Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and assigning containment levels for experiments which increase the host range and virulence of plant pathogens beyond that which occurs by natural genetic exchange.

With these modifications, the RAC accepted the proposals by a vote of fourteen in favor, none opposed, and three abstentions.

I accept these recommendations.

I–D. Proposal To Amend Section I–D–6 fo the Guidelines

In a letter of April 10, 1980, Dr. Irving Johnson of Lilly Research Laboratories proposed, among other changes, that Section I–D–6 of the Guidelines should be amended. The proposal would delete the second paragraph of Section I–D–6, which reads as follows:

> We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

The proposal was published for thirty days of comment in the **Federal Register** of April 30, 1980 [45 FR 28908].

No comments concerning this proposal were received during the thirty-day comment period.

The RAC discussed this proposal at the June 5–6, 1980 meeting. It was pointed out that the probability of escape does not necessarily increase with increasing scale. The RAC noted that the paragraph constitutes prefatory language and that its deletion would not change the containment standards of the Guidelines.

By a vote of fourteen in favor, one opposed, and one abstention, the RAC recommended that the second paragraph of Section I–D–6 of the Guidelines be deleted.

I accept this recommendation.

I–E. Proposal To Amend Portions of Section III–A–2–a, Viruses of Eukaryotes

In the 1980 Guidelines a level of biological containment requiring the use of an "HV1 host and a vector certified for use in an HV2 system" was substituted for "an EK1 host and a vector certified for use in an EK2 system." This level of biological containment is abbreviated as "HV1CV" in Tabe III and Footnote 40 of the 1980 Guidelines. At the December 6–7, 1979 meeting of the RAC, a working group was appointed to review the appropriateness of this level of biological containment. The working group proposed that HV2 biological containment be required for these experiments. Accordingly, a proposal to amend portions of Sections III–A–2–a, Viruses of Eukaryotes, was published in the **Federal Register** of January 31, 1980 [45 FR 7182].

The RAC discussed this proposal at the March 6–7, 1980 meeting. During the discussion, the RAC agreed that the "CV" nomenclature, developed for EK host-vector systems, did not seem appropriate for HV systems in general. However, the RAC felt that requiring the use of HV2 systems instead of HV1CV imposed an unnecessarily stringent level of containment. The RAC voted to publish for comment in the **Federal Register** a proposal to sustitute HV1 for HV1CV in all places in the Guidelines.

The proposal as published in the **Federal Register** of April 30, 1980 reads as follows:

> Substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(3)–(b) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(2)–(c), III–A–2–a–(1)–(b)–(1)–(b), III–A–2–a–(1)–(b)–(2)–(b), III–A–2–a–(2)–(a)–(1)–(b), III–A–2–a–(2)–(a)–(2)–(b), and III–A–2–a–(2)–(c)–(2)–(b). In Table III, whenever any one of the following three terms appears, ("HV1CV [40]," "HV1CV[40] or P3 + HV1," or "HV1CV[40] or P3 + HV1[36]") it would be changed to "HV1".

No comments were received during the thirty-day comment period. The RAC at its June 5–6, 1980 meeting, once again examined this issue. A motion to table failed by a vote of two in favor, nine opposed, and three abstentions. By a vote of nine in favor, and five opposed, the RAC accepted these proposed changes as published in the **Federal Register.**

I accept this recommendation.

I–F. Proposal To Amend Section III–B–3 of the Guidelines

Dr. Allan Campbell of Stanford University proposed the the RAC consider amending Section III–B–3 of the Guidelines , which currently read as follows:

> III–B–3. Non-HV1 systems. Containment levels for other classes of experiments involving non HV1 systems may be approved by the Director, NIH. [See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b)].
>
> In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 Containment. (2A)

Dr. Campbell stated that the P3 containment level was assigned to this type of experiment because of apprehensions that the category might contain some experiments for which that level was appropriate, rather than that all experiments in this category were perceived as requiring P3 containment. He proposed amending this section to "assert RAC's intention to review individual proposals in an open-minded manner with respect to realistic evaluation of possible hazard, and to discourage the inference that the P3 level, as it applies to a specific experiment, has been set on the basis of information about the organisms involved."

Dr. Campbell's proposed changes in the Guidelines were published in the **Federal Register** of April 30, 1980 [45 FR 28906] for thirty days of public comment, as follows:

> A. Section III–B–3, paragraph 2, would be amended to read:
>
> In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-receipient combinations (See Section IV–E–1–b–2–(f).
>
> B. A new Section IV–E–1–b–2–(f) would be added to the Guidelines as follows:
>
> IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (See Section III–B–3).

No comments were received during the thirty-day comment period. The RAC discussed this issue at the June 5–6, 1980 meeting. During the discussion, Dr. Campbell said that the term "non-pathogenic" refers to CDC Class 1 etiological agents. It was noted that the proposal simplifies procedures by removing the requirement that proposals to lower physical containment levels for specific donor-recipient combinations falling under Section III–B–3 be published in the **Federal Register** for public comment, prior to review by the RAC.

The RAC recommended acceptance of this proposal by a vote of thirteen in favor, none opposed, and four abstentions.

I accept this recommendation.

I–G. Proposal To Amend Section III–C–4 of the Guidelines

Dr. Milton Zaitlin proposed at the March 6–7, 1980 RAC meeting that Section III–C–4 of the Guidelines be amended to redefine a P1 greenhouse or growth chamber when certain infectious agents other than viruses are used as vectors.

Dr. Zaitlin noted that the description of a P1 greenhouse or growth chamber in Section III–C–3, Plant Viral Host-Vector Systems, recommends positive air pressure be maintained in order to exclude insects which might transmit viruses. However, in the conditions considered in Section III–C–4, i.e., Plant Host-Vector Systems other than Viruses, the propagules themselves (spores, mycelia, etc.) should be contained, and negative air pressure would be more appropriate.

He proposed that the following language be added at the end of Section III–C–4:

> and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules.

This proposal was published in the **Federal Register** of April 30, 1980 [45 FR 28906]. No comments were received during the thirty-day comment period.

The RAC considered this proposal at the June 5–6, 1980 meeting. The proposal was approved by a vote of fourteen in favor, and none opposed.

I accept this recommendation.

I–H. Request To Delete Language Requiring the Use of Cauliflower Mosaic Virus (CaMV) Mutants Lacking the Aphid Transmission Factor From Section III–C–3. Request for Permission To Transfer DNA Fragments From Aphid Transmissible to Aphid Nontransmissible CaMV

In a letter of January 17, 1980, Dr. Robert J. Shepherd of the University of California at Davis requested that paragraph four of Section III–C–3 of the Guidelines be deleted. This paragraph reads as follows:

> The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid transmission factor.

Dr. Shepherd suggested that it is inconsistent to restrict studies with cauliflower mosaic virus to non-insect transmissible strains when no such restriction is imposed on studies with bean golden mosaic virus and related viruses, the only other known group of DNA plant viruses. Dr. Shepherd noted that, under the P1 containment conditions specified in the Guidelines, cauliflower mosaic virus is easily confined to a growth chamber or greenhouse. He added that CaMV has a very restricted host range and is neither seed nor pollen transmitted.

Dr. Shepherd also requested permission to introduce DNA segments from aphid transmissible strains to non-aphid transmissible strains of CaMV in order to study the factors determining aphid transmissibility.

A notice outlining Dr. Shepherd's request appeared in the **Federal Register** of April 30, 1980 [45 FR 28906] for thirty days of public comment.

No comments were received during the thirty-day comment period.

The RAC discussed Dr. Shepherd's proposal at the June 5–6, 1980 meeting. By a vote of ten in favor, none opposed, and four abstentions, the RAC recommended that paragraph four of Section III–C–3 be deleted from the Guidelines. By a vote of eleven in favor, none opposed, and three abstentions, the RAC recommended approval of Dr. Shepherd's request for permission to introduce DNA segments from aphid transmissible strains to the genome of non-aphid transmissible strains of CaMV.

I accept these recommendations.

I–I. Status of Variola and Whitepox Viruses

At the March 6–7, 1980 meeting of the RAC, the suggestion was made that any mention in the Guidelines of the variola viruses be marked with a symbol so that reference could be made to World Health Organization policy regarding worldwide efforts at eradicating these viruses.

Accordingly, it was proposed that Appendix B, *Classification of Microorganisms on the Basis of Hazard,* be amended so that each mention of Alastrim, Smallpox and Whitepox will be marked with a symbol. With the concurrence of the Center for Disease Control, the following footnote, to be added to Appendix B, appeared in the **Federal Register** of April 30, 1980 [45 FR 28906] for thirty days of comment:

> All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

During the thirty-day comment period, no comments were received. The RAC at its June 5–6, 1980 meeting recommended that the marking of any mention of Alastrim, Smallpox and Whitepox in Appendix B was appropriate, and approved the proposed language of the footnote by a vote of seventeen in favor, and none opposed.

I accept this recommendation.

I–J. Transfer of DNA Segments Cloned in Saccharomyces Cerevisiae Host-Vector Systems to Higher Plants

Dr. Jane Setlow of Brookhaven National Laboratory suggested that the Guidelines include a general provision for cloning plant DNA in *Saccharomyces cerevisiae* and subsequently returning the cloned DNA to the plant. The following proposed amended language of the first sentence of Section III–C–7–b appeared in the **Federal Register** of April 30, 1980 [45 FR 28906]:

> III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under appropriate containment conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study [2A].

During the thirty-day comment period, no comments were received.

The RAC discussed this proposal at the June 5–6, 1980 meeting. The proposal was accepted by a vote of twelve in favor, none opposed, and six abstentions.

I accept this recommendation.

The language of the **Federal Register** announcement of April 30, 1980 [45 FR 28906] differed from the language of the revised Guidelines promulgated on January 29, 1980 [45 FR 6738]; the latter specify "P1 conditions," while the former said "appropriate containment conditions." The phrase "appropriate containment" had been published in the April 30, 1980 **Federal Register** announcement as the proposal to include laboratory strain *S. cerevisiae* host-vector systems under Section III–O had not yet been considered by the RAC and the NIH. The latter proposal is now being accepted, and new language for Section III–O is being promulgated in this document. As laboratory strain *S. cerevisiae* host-vector systems will henceforth be included under Section III–O, the language stipulating P1 containment conditions, being more precise, will be used in Section III–C–7–b.

I–K. Request for Consideration of Appropriate Containment Conditions

Dr. Paul S. Sypherd of the University of California, Irvine, requested in a letter of May 13, 1980 that the RAC evaluate containment conditions appropriate to the return of *Mucor racemosus* DNA cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In Addition, Dr. Sypherd requested permission to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae* sequences. Dr. Sypherd noted that *Mucor* species in general, and *M. racemosus* in particular, are ubiquitous. *M. racemosus* is globally distributed and is found in air, in soil, on fruits, and in animal dung. He added that *M. racemosus* is nonpathogenic and produces no known toxins. *M. racemosus* is not known to mate with other species of *Mucor*, and mating within the species occurs at a very low frequency under precisely defined conditions. A **Federal Register** announcement of Dr. Sypherd's request appeared on April 30, 1980 [45 FR 28908]

During the thirty-day comment period, no comments were received.

During the discussion of this issue at the June 5–6, 1980 meeting, it was noted that certain species of *Mucor* can cause mycoses in medically compromised humans. It was thus suggested that the principal investigator be alerted to the pathogenic potential of certain *Mucor* species.

By a vote of fourteen in favor, none opposed, and four abstentions, the RAC recommended acceptance of Dr. Sypherd's proposal at the P2 containment level.

I accept this recommendation.

I–L. Cloning of Foot and Mouth Disease Virus—Stage II

The RAC at its June 5–6, 1980 meeting considered the request from scientists at the Plum Island Animal Disease Center to remove clones containing sequences equivalent to a portion of the genome of the Foot-and-Mouth Disease (FMD) Virus from Plum Island for further development at Genentech, Inc., of South San Francisco, California. This is Stage II of a cooperative recombinant DNA project by scientists at both institutions to clone segments of the Foot-and-Mouth Disease Virus for the purpose of developing a vaccine derived from the VP$_3$ protein encoded by the virus. Stage I of the project, involving the construction and analysis of a gene bank of plasmids containing FMD sequences, had been completed at Plum Island. In accord with the recommendations of the RAC at its December 6–7, 1979 meeting, and the approval of the Director, NIH, the investigators submitted data to demonstrate that the clones to be removed from Plum Island to Genentech, Inc. are noninfective, and contain sequences in aggregate less than the full genome of the FMD virus.

Announcement that the RAC would review the request to remove clones from Plum Island and would recommend containment levels for the work at Genentech, Inc., appeared in the **Federal Register** of April 30, 1980. No comments were received during the thirty-day comment period.

Information on the characterization of the inserted DNA sequences was provided to the RAC to support the RAC's stipulation that "clones to be approved for removal from Plum Island shall not contain among them collectively or individually, the full genome of the FMD virus." (A full discussion of the RAC's previous recommendations appears in 45 FR 3552.) The RAC further considered the evidence presented which demonstrated that the clones lack infectivity. The hybridization and restriction analysis data for characterization were found satisfactory.

On the basis that the 17 clones described in the submission were well characterized, lacked infectivity, and represent, in aggregate, only 75% of the FMD viral genome, a motion was passed to recommend approval for removal of these clones from Plum Island by a vote of twelve in favor, two opposed, and four abstentions.

A motion to permit work with the clones at Genentech, Inc. under P1 + EK1 conditions passed by a vote of eleven in favor, one opposed, and five abstentions.

I accept these recommendations.

I–M. Lowering of Containment Levels for Characterized Clones or Purified DNAs by Institutional Biosafety Committees

The RAC at its March 6–7, 1980 meeting voted nine in favor, one opposed, with three abstentions, that there be published in the **Federal Register** for comment, and brought back to the RAC at its June 5–6, 1980 meeting, proposed revisions of the Guidelines to permit a single-step reduction in physical or biological containment for characterized clones or purified DNA, by the local Institutional Biosafety Committee (IBC), without a requirement for NIH approval. Accordingly, the following proposed changes in the Guidelines were published in the **Federal Register** of April 30, 1980:

A. *Section III–A–3–a is to be amended as follows:*

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may decrease one step in physical containment [P4→P3; P3→P2; P2→P1] while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment [HV3→HV2; HV2→HV1] while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1 + HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)–(e).)

B. *Section III–A–3–b is to be amended as follows:*

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.

Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III—A—3—b to levels below P1+HV1, or reduction of containment levels by more than one step, which also require prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–3–(e).)

C. *Section IV–D–1–c–(3) is to be amended as follows:*

IV–D–1–c–(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3).

D. *The Note in Section IV–D–3–b is to be amended as follows: (Additional language in Italics).*

Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other example are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. *Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been pruified and are judged to be free of harmful sequences (See Section III–A–3–a) and for (ii) clones that have been characterized and judged to*

> *be free of harmful sequences (see Section III–A–3–b).* It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

No comments were received during the thirty-day comment period. These proposed changes of the Guidelines were considered by the RAC at its June 5–6, 1980 meeting. The RAC recommended the changes by a vote of fifteen in favor, none opposed, and no abstentions.

I accept the proposed changes.

I-N. Request for Permission To Transform Saccharomycopsis Lipolytica With E. Coli/S. Cerevisiae Hybrid Plasmids

In response to a request to approve the transformation of the yeast *Saccharomycopsis lipolytica* with defined *E. coli/Saccharomyces cerevisiae* hybrid plasmids the following notice appeared in the April 30, 1980 **Federal Register:**

> "Dr. David M. Ogrydziak of the University of California, Davis, requests permission to attempt to transform the yeast *Sacchromycopsis lipolytica* with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and to use the hybrid plasmids for shotgunning *S. lipolytica* DNA in *E. coli* and returning the DNA to *S. lipolytica.*

During the thirty-day comment period there were no comments. The RAC discussed the proposal during its June 5–6, 1980 meeting. It was noted that *S. lipolytica* is not a pathogen, and does not colonize animals. A Motion to approve these experiments at the P1 level of containment was passed by a vote of fifteen in favor, none opposed, and one abstention.

I accept this recommendation.

I–O Request for Use of Conjugative Plasmids and Transducing Phages in an Escherichia Coli K–12 Host.

The following notice was published in the **Federal Register** of April 30, 1980:

> Dr. A. I. Bukhari of the Cold Spring Harbor Laboratory has requested permission from the RAC to employ conjugative plasmids or transducing phages in recombinant DNA experiments involving *Escherichia coli* DNA when a small fragment of heterologous DNA is used as a linker. In the specific request, Dr. Bukhari requests approval to use a fragment of Adenovirus 2 DNA as linker DNA.

No comments were received during the thirty-day comment period.

In the RAC discussion of the request at its June 5–6, 1980 meeting, it was noted that the small Adenovirus DNA linker segments of 1 kb size were not derived from the transforming region and could, therefore, be treated as inert DNA. The motion recommending approval of this request passed by a vote of fourteen in favor, none opposed, and one abstention.

I accept this recommendation.

II. Summary of Major Actions Under Guidelines

II–A. Amendment of Section I–D–1

Section I–D–1 is amended to read:

> I–D–1. Formation of recombinant DNAs derived from pathogenic organisms classified [1] as Class 4 or 5 or from cells known (2A) to be infected with such agents, regardless of the host-vector system used.

II–B. Deletion of Section I–D–3

Section I–D–3 is deleted. Substitute for the current wording, the word "deleted."

II–C. Amendment of Section I–D–6 of the Guidelines

Delete the second paragraph of Section I–D–6, which reads as follows:

> We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

II–D. Amendment of Section I–E

Section I–E is amended to read:

> I–E. Exemptions. It must be emphasized that the following exemptions [4] are not meant to apply to experiments described in Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms [1] or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5.

II–E. Amendment of Section III

After the last sentence of Section III, "The use of higher levels of biological containment * * * for the purposes of the experiment," the following new paragraph is to be added:

> Experiments involving recombinant DNA from Class 3 organisms [1] or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section IV–E–1–b–2–e).

II–F. Amendment of Section III–O of the Guidelines

Section III–O of the Guidelines is amended to read as follows: [This amended language combines (1) the language of the proposal to include *Saccharomyces cerevisiae* host-vector systems under Section III–O, (2) the language of the proposal to include Fl phages under Section III–O, and (3) a paragraph pertaining to CDC Class 3 agents and certain plant pathogens.]

> III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).
>
> Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).
>
> Experiments using *E. coli* K–12 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–3–1–b–2–e).
>
> Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 hostvector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid of Fl bacteriophages or non-conjugative plasmids [49] shall be used as vestors. For experiments in *S. cerevisiae*, laboratory strains shall be used]. For these experiments no Memorandum of Understanding and Agreement (MUA) as described in Section IV–D–1–c need be submitted, nor is any registration with NIH necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, and (c) the hosts and vectors to be used. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.
>
> Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).
>
> For those not on Appendix A list but which exchange genetic information [35] with *E.*

coli, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or intergrated, or generalized transducing phages."

II–G Amendment of Section III–A–2–a, Viruses of Eukaryotes

Substitute the term "an HV1 host-vector" or "an HV1 host and a vector certified for use in an HV2 system" in Section III–A–2–a–(1)–(a)–(3)–(b) and substitute the term "an HV1 host-vector" for "an HV1 host and a vector certified for use in an HV2 system, or P3 + HV1," in Sections III–A–2–a–(1)–(a)–(2)–(c), III–A–2–a–(1)–(b)–(1)–(b), III–A–2–a–(1)–(b)–(2)–(b), III–A–2–a–(2)–(a)–(1)–(b), III–A–2–a–(2)–(a)–(2)–(b), and III–A–2–a–(2)–(c)–(2)–(b). In Table III, whenever any one of the following three terms appears, ("HV1CV[40]"; "HV1CV[40] or P3 + HV1;" or "HV1CV[40] or P3 + HV1[38]"). substitute "HV1."

II–H. Section III–A–3–a Is Amended as Follows

III–A–3–a. *Purified DNA Other Than Plasmids, Bacteriophages, and Other Viruses.* The information of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences has been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment [42]. The containment may be decreased one step in physical containment [P4→P3; P3→P2; P2 P1] while maintaining the biological containment specified for the shotgun experiement, or one step in biological containment [HV3→HV2; HV2→HV1] while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1 + HV1, which requires prior NIH approval. (See Sections IV–D–1–c and IV–E–1–b–(3)(e).)

II–I. Section III–A–3–b Is Amended as Follows

III–A–3–b. *Characaterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3) experiments involving this recombinant DNA may be carried out under lower containment conditions.

Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone dervied from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III-A-3-b to levels below Pl + HVl, or reduction of containment levels by more than one step, which also requires prior NIH approval. (See Sections IV-D-1-c and IV-E-1-b-3-(e).)

II-J Amendment of Section III-B-3

Section III-B-3, paragraph 2 is amended to read:

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment [2A]. Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations (See Section IV-E-1-b-2-(f)).

II-K. Amendment of Section III-C-3 of the Guidelines

Delete paragraph four of Section III-C-3, which reads as follows:

The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid trasmission factor.

II-L. Amendment of Section III-C-4 of the Guidelines

Paragraph two of Section III-C-4 is amended by changing the end of the paragraph from "* * * work." to "* * * work, and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules."

II-M. Amendment of Section III-C-7-b of the Guidelines

Section III-C-7-b is amended to read as follows:

III-C-7-b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I-D] which has been cloned and propagated in *E. coli* or *S. cerevisiae under P1 conditions, may be transferred with the E. coli* or *S. cerevisiae* vector used for cloning to any high plant organisms (angiosperms and Gymnosperms) and propagated under condition of physical containment comparable to Pl and appropriate to the organism under study (2A). Intact plants or propagative plant parts may be grown under P1 conditions described under Section III-C-3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis (45).

II-N. Section IV-D-1-c-(3) Is Amended as Follows

IV-D-1-c-(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III-A-3).

II-O. Amendment of Section IV-D-3-b

The note in Section IV-D-3-b is amended as follows:

Note.—Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been purified and are judged to be free of harmful sequences (See Section III-A-3-a) and for (ii) clones that have been characterized and judged to be free of harmful sequences (see Section III-A-3-b). It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

II–P. Addition of Section IV–E–1–b–2–(e)

A new Section IV–E–1–b–2–(e) is added as follows:

IV–E–1–b–2–(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms [1] and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens beyond that which occurs by natural genetic exchange.

II–Q. Addition of Section IV–E–1–b–2–(f)

A new Section IV–E–1–b–2–(f) is added a follows:

IV–E–1–b–2–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (See Section III–B–3).

II–R. Amendment of Footnote 2, Section V

Section V, footnote 2, is amended to read:

2. For experiments using Vesiculer Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

II–S. Amendment of Footnote 38, Section V

The second sentence of footnote 38, Section V is amended to read:

(As noted in the Prohbition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

II–T. Status of Variola and Whitepox Viruses

A new footnote is added to Appendix B of the Guidelines as follows:

* * * All activities, including storage of variola and whitepox are restricted to the single national facility (World Health Organization (WHO) Collaborating Center

for Smallpox Research, Center for Disease Control, in Atlanta).

Each mention of Alastrim, Smallpox and Whitepox in Appendix B should be followed by the symbol * * *.

II–U. Amendment of Appendix D

Appendix B is amended to read:

As noted above at the beginning of Section III–A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III–A. Those so classified as of publication of these Revised Guidelines are listed below.

HV1[*]—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:

(1) inl (inositolless) strains 37102, 37401, 46316, 64301 and 89601.

(2) csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—Asporogenic mutant derivatives *B. subtilis*. The *B. subtilis* HV1 derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223. *B. subtilis* strain RUB 331 has been certified as the host component of HV1 systems based on these plasmids.

II–V. Amendments of Appendix E

The following sections are added to Appendix E:

- Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.
- *Sacchromycopsis lipolytica* may be used as a host for transformation with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and the hybrid plasmids may be used for cloning *S. lipolytica* DNA in *E. coli* and returning the cloned DNA to *S. lipolytica*.
- Conjugative plasmids or transducing phages may be employed in recombinant DNA experiments when employing *E. coli* as host when a small defined segment of Adenovirus 2 DNA is employed as linker DNA.
- Permission is granted to introduce DNA segments from aphid transmissible strains into non-aphid transmissible strains of Cauliflower mosaic virus in order to study the factors determining aphid transmissibility.
- Permission is granted to return *Mucor racemosus* DNA which has been cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In addition, permission is granted to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae* sequences. These manipulations may be performed under P2 conditions.
- Many experiments utilizing *E. coli* or *S. cerevisiae* host-vector systems are now covered by Section III–O of the Guidelines. This section states, in part, that "* * * experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host/vector system * * *"

While the Guidelines no longer specifiy the use of EK2 or *S. cerevisiae* HV2 systems, investigators may wish to employ these systems in specific instances. The currently certified EK2 and HV2 systems are:

HV2—The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32 and YIp33.

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi–1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 Systems based on bacteriophage lambda:

Vector	*Host*
λ gt*WES*. λ B'	DP50*supF*
λ gt*WES*. λ B*	DP50*supF*
λ gt*ZJvir*. λ B'	*E. coli* K–12
gtALO. λ B	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Additional Announcements of the Director, NIH

In addition, two further changes are being promulgated in order to clarify the intent of the language in two sections of the Guidelines.

I. Addition of Subheading to Section I–D

A new subheading, "I–D (1–6)," is to be added to the penultimate paragraph in Section I–D to emphasize that this paragraph applies to I–D–1 through I–D–6. The amended paragraph reads as follows:

I–D (1–6). Experiments in Categories I–D–1 to I–D–6 may be excepted (4) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment (See Section IV–E–1–b–(1)–(e).)

II. Amendment of Appendix E, Item 6

Item 6 in Appendix E is amended to read as follows:

Streptomyces coelicolor can be used as a host for the cloning of DNA derived from *B. subtilis*, *E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as a vector any plasmid indigenous to Streptomyces coelicolor or able to replicate in that host by natural biological mechanisms.

Dated: July 18, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that Circular.

[FR Doc. 80–22366 Filed 7–28–80; 8:45 am]

BILLING CODE 4110–08–M

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[*] These follow the assigned containment levels as specified in the subsections of Section III–A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3 + HV1 or P2 + HV2 rather than the levels given in the subsections of Section III–A.

federal register

Thursday
August 21, 1980

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the Linden Hill Hotel, Linden Room, 5400 Pooks Hill Road, Bethesda, Maryland 20014, on September 25 1980, from 9:00 a.m. to recess at approximately 6:00 p.m., and on September 26, 1980, from 8:30 a.m. to 5:00 p.m. This meeting will be open to the public on September 25 from 9:00 a.m. to recess at approximately 6:00 p.m., and on September 26 from 8:30 a.m. to approximately 3:00 p.m. to discuss:

Amendment of Guidelines
Procedures for review of proposals for large scale production
Voluntary compliance with guidelines
Institutional Biosafety Committees
Exceptions to prohibitions
Exemptions for organism that exchange genetic information
Review of large-scale proposals
E. coli K–12 host-vector systems
Host-vector systems other than *E. coli* K–12
NIH risk-assessment plan
Review of protocols for required containment levels
Other matters requiring necessary action by the Committee.

Attendance by the public will be limited to space available.

In accordance with provisions set forth in Section 552b(c)(4), Title 5, U.S. Code and Section 10(d) of P.L. 92–463, the meeting will be closed to the public for approximately two hours for the review, discussion and evaluation of proposal(s) from a commerical concern(s) for scale-up of recombinant DNA experiments. It is anticipated that this will occur on September 26, from approximately 3:00 p.m. until adjournment. The proposal(s) and the discussion could reveal confidential trade secrets or commerical property such as patentable material.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 4A52, telephone 301–496–6051, will provide materials to be discussed at the meeting, rosters of committees members and substantive program information. A summary of the meeting will be available at a later date.

Dated: August 14, 1980.
Donald S. Fredrickson, M.D.,
Director, NIH.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcement the number and title of affected individual programs for the guidance of the public because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that circular.

[FR Doc. 80-25289 Filed 8-20-80; 8:45 am]

BILLING CODE 4110-08-M

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under NIH guidelines for research involving recombinant DNA molecules.

SUMMARY: This notice sets forth proposals for actions to be taken under the 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules [**Federal Register** of January 29, 1980 (45 FR 6724)]. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its September 25–26, 1980, meeting, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by September 22, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 4A52, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban or Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following changes and amendments under the Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724), as well as actions under these Guidelines.

1. *Requests for permission to transform chlamydomonas reinhardi with E. colisaccharomyces cerevisiae plasmids.* Dr. John Carbon of the University of California, Santa Barbara, and Dr. Stephen Howell, University of California, San Diego, request permission to introduce *Escherichia coli-Saccharomyces cerevisiae* hybrid plasmids containing defined segments of DNA into the unicellular flagellate *Chlamydomonas reinhardi* under P2 physical containment conditions.

2. *Request for permission to transform candida albicans with E. Coli-S. Cerevisiae plasmids.* Dr. P. T. Magee of Michigan State University, and Dr. W. LaJean Chaffin of Texas Tech University, have requested consideration of the appropriate containment level for the return of *Candida albicans* DNA to the host of origin. The *Candida albicans* DNA will be cloned in *E. coli* K–12 or in *Saccharomyces cerevisiae* employing a hybrid plasmid vector derived from *E. coli* K–12–*S. cerevisiae* or the yeast 2 micron plasmid.

3. *Proposal to introduce genes cloned in E. Coli K–12 into Arabidopsis plants through the use of Agrobacterium tumefaciens carrying an E. Coli/Ti hybrid plasmid vector.* Employing the following protocol, Dr. Donald J. Merlo of the University of Missouri-Columbia, requests permission to introduce genes into *Arabidopsis thaliana:*

(a) A hybrid plasmid vector, constructed from the *E. coli* plasmid pBR325 and the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, will be cloned in *E. coli K–12.*

(b) *Arabidopsis* DNA will be introduced into the *E. coli*/Ti hybrid plasmid and cloned in *E. coli* K–12.

(c) The thiamine gene of *E. coli* will be introduced into the *E. coli*/Ti vector carrying *Arabidopsis* DNA and cloned in *E. coli* K–12.

(d) The hybrid plasmid into which *Arabidopsis* DNA and the thiamine gene have been ligated will be transformed into *Agrobacterium tumefaciens*.

(e) *Agrobacterium tumefaciens* will be used to introduce the *E. coli*/Ti plasmid vector carrying the *E. coli thiamine gene and Arabidopsis* DNA into *Arabidopsis* plants.

According to Section III–O of the Guidelines, steps, (a), (b) and (c) of the protocol may be performed under P1 containment; according to Appendix E, steps (d) and (e) may be conducted under P3 containment. Dr. Merlo requests permission to perform steps (d) and (e) at P1 or P2 containment. He suggests that the DNA combination to be generated could occur in nature, albeit at very low frequency.

4. *Request for certification of a Bacillus subtilis strain as the host-vector component of an HV2 host-vector system.* On March 28, 1980, Dr. William F. Burke, Jr., of Arizona State University, requested certification of *Bacillus subtilis* strain ASB298 as the host component of an HV2 host-vector system. Dr. Burke's proposal was evaluated by a working group and subsequently presented to the RAC at the June 5–6, 1980 meeting. The RAC discussed the characteristics of a *Bacillus subtilis* HV2 system and recommended that consideration of the proposal be deferred until additional information concerning transfer of recombinant DNA through transformation was received. The RAC will continue the evaluation of additional information received on strain ASB298 at the September meeting.

5. *Request for HV1 certification of a Bacillus stearothermophilus derived plasmid vector in Bacillus subtilis.* Dr. David B. Wilson of Cornell University, requests HV1 certification of a host-vector system based on a *Bacillus subtilis* host and a plasmid isolated from *Bacillus stearothermophilus*.

6. *Proposed revision of subsections of section III–C–1–e.* A notice appeared in the **Federal Register** of January 31, 1980 concerning proposed revision of Section III–C–1–e, and its subsections. It was recommended that Sections II–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e–(1)–(b), of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The RAC, at its March 6–7, 1980 meeting, recommended adoption of Sections III–C–1–e, III–C–1–e–(1), and III–C–e–(1)–(a) as published in the **Federal Register** of January 31, 1980, with certain modifications in Section III–C–1–e–(1)–(a).

The Director, NIH, accepted this recommendation and promulgated the following sections in the **Federal Register** of April 14, 1980:

"III–C–1–e. All Viral Vectors.

III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:

III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses form a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragment of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

At its March 6–7, 1980 meeting, the RAC voted to defer consideration until the June 5–6, 1980 meeting of the new Sections III–C–1–e(1)–(b) and III–C–1–e–(1)–(c) as proposed in the **Federal Register** of January 31, 1980, and requested that a working group develop additional information. Accordingly, a working group met on May 13, 1980 during the annual meeting of the American Society for Microbiology in Miama Beach, Florida. The report of the working group was considered briefly at the June 5–6 RAC meeting and will be considered again by the RAC at its September 25–26, 1980 meeting.

The Working Group discussed the question of the appropriate containment conditions for experiments involving recombinant DNA molecules containing less than two-thirds of the genome of any eukaryotic virus which may be rescued with helper virus. On the basis of the consensus of the virologists, the following recommendation was proposed, as a revision of Section III–C–1–e–(1)–(b) of the Guidelines

"III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents (1)."

7. *Procedures for review of large-scale experiments.* Section I-D-6 of the Guidelines prohibits "large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established."

Section IV-E-1-b-(3)-(d) of the Guidelines states that the Director, NIH, is responsible for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Part VI of the Guidelines, "Voluntary Compliance," encourages institutions not otherwise covered by the Guidelines to follow the standards and procedures set forth in the Guidelines.

At its September 1979 meeting, the RAC adopted the following procedures to be followed by applicants proposing to exceed the 10-liter limit:

"Application Procedures For Large-Scale Recombinant DNA Experiments

1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

b. A statement of the rationale for wishing to exceed the 10-liter limit.

c. A specification of the total volume of the fermenter to be used.

d. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

e. A description of the applicant's laboratory practices, containment equipment, and facilities relevant to the containment of large volumes of culture.

f. Evidence of the applicant's or applicant institution's previous experience in handling large volumes of culture. Applicants should exhibit knowledge of state-of-the-art procedures for working with large volumes of microorganisms.

g. A description of procedures to be employed for the inactivation and disposal of large volumes of culture.

h. A description of procedures for containing and inactivating accidental spills, should they occur.

2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

4. Following review and approval by the RAC, each request shall be

submitted to the Director, NIH, for final review.

5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

At the last few RAC meetings, there have been extensive discussions of the role of the RAC and NIH in the review of large-scale proposals submitted by industry. (Minutes of RAC meetings are available from ORDA.) At the June 1980 meeting, the RAC passed a motion by a vote of seventeen to zero with one abstention that the following proposal be published in the **Federal Register** for consideration at the September, 1980 meeting:

> The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. The RAC will determine if a given recombinant DNA-containing strain is rigorously characterized and the absence of harmful sequences established. Such a determination shall include specification of a containment level (P–LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industrial scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC.

If the above proposal is accepted, it would have the effect of changing the application procedures to read as follows:

"Application Procedures for Large-Scale Recombinant DNA Experiments

1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

b. A statement of the rationale for wishing to exceed the 10-liter limit.

c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

d. Specification of the P–LS level proposed to be used as defined in the NIH *Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules.* (**Federal Register**, April 11, 1980).

2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

4. Following review and approval by the RAC, each request shall be submittd to the Director, NIH, for final review.

5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provision of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

8. *Proposed changes in registration requirements.* Dr. Maxine Singer, National Cancer Institute, National Institutes of Health, has proposed a series of changes in the administrative requirements specified by the Guidelines. In brief, Dr. Singer's proposal eliminates NIH review, registration, and approval for all experiments assigned containment conditions in the Guidelines. The reasons for the proposed changes are discussed in a letter to ORDA.

Specifically, the proposal would revise several sections of the Guidelines. The proposed revisions are discussed below.

A. *Section III.* Section III of the Guidelines would be amended to read as follows:

"III. *Containment Guidelines for Covered Experiments.*

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Containment guidelines for permissible experiments are given in Part III. For these experiments no registration with NIH is necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene in the cloning vehicle and if so, what protein, and (e) the containment conditions specified by these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals but such review is not required prior to initiation of experiments except as noted in Sections III–A–3–a, III–A–3–b, or for experimgnts where containment procedures are not explicitly described in these Guidelines.

Changes from the levels specified in Part III for specific experiments (or the assignment of levels to experiments not explicitly considered here) may not be instituted without the express approval of the Director, NIH. (See Sections (IV–E–1–b(1)–(a), IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)(b).)

In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimal for the experiment designated. The use of higher levels of biological containment (HV3>HV2>HV1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

Experiments involving recombinant DNA from Class 3 organisms [1] or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other expriments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section IV–E–1–b–2–(e))."

B. *Section III–O.* Section III–O of the Guidelines would be amended to read as follows:

"III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Experiments using *E. coli* K–12 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–E–1–b–2–(e)).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host-vector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or nonconjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used].

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known phsyiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information [35] with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a nonconjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages."

C. *Section III–A–3–a.* The last sentence of Section III–A–3–a would be amended to read as follows:

"IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1+HV1, which requires prior consultation with NIH."

D. *Section III–A–3–b.* The last sentence of the second paragraph of Section III–A–3–b would be amended to read as follows:

"IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1+HV1, or reduction of containment levels by more than one step, which requires prior consultation with NIH."

E. *Section IV–C–3.* Section IV–C–3 would be deleted. This Section currently reads as follows:

"IV–C–3. "Memorandum of Understanding and Agreement" or "MUA" is a document that (i) provides to NIH or other Federal funding agency an Institution's certification that the recombinant DNA research project complies with the NIH Guidelines and (ii) contains other essential data as required in the Administrative Practices Supplement."

F. *Section IV–D–1–c.* Sections IV–D–1–c and IV–D–1–c–(1) through IV–D–1–c–(5) would be deleted. These Sections currently read as follows:

"IV–D–1–c. Submit, for each recombinant DNA project that meets with its approval, a Memorandum of Understanding and Agreement (MUA) to the funding agency for approval and registration. (Note: No MUA is required for experiments described in Section III–O). All projects, however, can proceed upon IBC approval (before submission of the MUA to the funding agency) except for the following, which require prior approval by NIH (or other funding agency designated by NIH for this purpose):

"IV–D–1–c(1). Projects for which containment levels are not specified by the Guidelines or NIH,

IV–D–1–c–(2). Projects requiring P4 containment,

IV–D–1–c–(3). Certain reductions of containment levels for characterized or purified DNA preparations or clones (see Section III–A–3),

IV–D–1–c–(4). The first project conducted in a facility at P3 containment, or,

IV–D–1–c–(5). The first project conducted by an Institution.

Note.—The MUA shall be submitted to the funding agency within 30 days of the IBC approval. If the funding agency does not routinely register recombinant DNA projects with NIH, the MUA must be submitted to NIH as well as to the funding agency. Authority to submit MUAs (or addenda) for which prior approval is not required may be delegated to the IBC chairperson. All MUAs that require NIH approval before the work can proceed shall be submitted to the NIH by the institutional official to whom the IBC is responsible."

G. *Section IV–D–1–d.* Section IV–D–1–d would be deleted. This section currently reads as follows:

"IV–D–1–d. Take appropriate action to bring protocols into compliance when advised by NIH or other funding agency that IBC-approved projects do not conform to standards set forth in the Guidelines. This responsibility may be delegated to the IBC. (See Administrative Practices Supplement for further details)."

H. *Section IV–D–2–f.* Section IV–D–2–f would be amended to read as follows:

"IV–D–2–f. Central to implementation of the Guidelines is the review of experiments by the IBC. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement."

I. *Section IV–D–2–h.* Reference to MUAs would be deleted in Section IV–D–2–h. The revised Section would read as follows:

"IV–D–2–h. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public (e.g., reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to HIH both the comments and the IBC's response."

J. *Section IV–D–3–a.* Section IV–D–3–a would be amended to read as follows:

"IV–D–3–a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. (See Administrative Practices Supplement for prior NIH approval requirements.) This review shall include:"

K. *Section IV–D–3–b.* Section IV–D–3–b would be amended to read as follows:

"IV–D–3–b. Notifying the Principal Investigator (PI) of the results of their review."

The "Note" following IV–D–3–b would be deleted.

L. *Section IV–D–3–f.* Section IV–D–3–f would be renumbered to IV–D–3–g, and a new Section IV–D–3–f would be added as follows:

"IV–D–3–f. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH, (with the advice of the RAC when required) establishes the containment requirements."

M. *Amendment of Section IV–D–5–a–(1).* Section IV–D–5–a–(1) would be amended to read as follows:

"IV–D–5–a–(1). Initate or modify no recombinant DNA research subject to the Guidelines until the Institutional Biosafety Committee (IBC) has been appropriately notified and all other requirements of the Guidelines and the Administrative Practices Supplement (APS) have been met. Modify containment and experimental protocol according to recommendations of the IBC."

N. *Proposed Section IV–D–5–b–(4).* A new Section IV–D–5–b–(4) would be added as follows:

"IV–D–5–b–(4). Petition NIH for recommendations regarding containment for experiments requiring case-by-case review."

O. *Proposed Section IV–D–5–b–(5).* A new Section IV–D–5–b–(5) would be added as follows:

"IV–D–5–b–(5). Petition NIH for recommendations regarding containment for experiments not covered by the Guidelines."

P. *Amendment of Section IV–D–5–c–(3).* Section IV–D–5–c–(3) would be amended to read as follows:

"IV–D–5–c–(3). Submit the initial research protocol (and also subsequent changes—e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval;"

Q. *Amendment of Section IV–D–5–d.* The heading of Section IV–D–5–d would be amended to read:

"IV–D–5–d. *PI Responsibilites Prior to Initiating the Research.*"

R. *Amendment of Section IV–D–5–e.* The heading of Section IV–D–5–e would be amended to read:

"IV–D–5–e. *PI Responsibilities During the Conduct of the Research.*"

S. *Section IV–D–5–e–(6).* A new Section, IV–D–5–e–(6), would be added to read as follows:

"IV–D–5–e–(6). Initiating no recombinant DNA research for which containment is not specified by the Guidelines until approval is received from the IBC."

T. *Deletion of Section IV–E–1–b(3)–(e).* Section IV–E–1–b(3)–(e) would be deleted. This section currently reads as follows:

"IV–E–1–b–(3)–(e). Lowering containment levels for characterized clones or purified DNA (See Sections III–A–3–a and –b, and Footnotes 3 and 41);"

U. *Deletion of Sections IV–E–3–b, IV–E–3–c, and IV–E–3–c–(1) through IV–E–3–c–(3).* Sections IV–E–3–b, IV–E–3–c, IV–E–3–c–(1), IV–E–3–c–(2), and IV–E–3–c–(3) would be deleted. These sections currently read as follows:

"IV–E–3–b. Registration of recombinant DNA projects; and

IV–E–3–c. Review of Memoranda of Understanding and Agreement (MUAs), and approval of those that conform to the Guidelines. In so doing, ORDA shall:

IV–E–3–c–(1). Conduct an independent evaluation of the containment levels required for the research covered by these Guidelines;

IV–E–3–c–(2). Determine whether the physical and biological containment levels approved by the IBC are in accordance with the requirement of the Guidelines;

IV–E–3–c–(3). Notify Institutions and the IBC chairperson in a timely fashion when MUAs (including changes in ongoing projects) do not conform to the Guidelines, and inform them of corrective measures to be taken;"

V. *Deletion of Section IV–E–4–a.* Section IV–E–4–a would be deleted. This Section currently reads as follows:

"IV–E–4–a. Awarding no grant or contract involving recombinant DNA techniques unless a properly executed MUA has been received;"

W. *Deletion of Sections IV–F–1 and IV–F–2.* Sections IV–F–1 and IV–F–2 would be deleted. These sections currently read as follows:

"IV–F–1. *Required Registration.* Institutions receiving NIH funds for recombinant DNA projects shall inform NIH of all recombinant DNA projects at the Institution. A non-NIH project, after approval by the Institutional Biosafety Committee, shall be registered with NIH within 30 days of initiation. Applications for NIH projects must be accompanied by a Memorandum of Understanding and Agreement (MUA)."

"For information on MUAs or equivalent documents that must be submitted for registration of recombinant DNA projects, see the Administrative Practices Supplement (APS)."

"IV–F–2. *Federal Agency Registration.* Institutions at which recombinant DNA research projects funded by other Federal agencies are conducted need not register such projects with NIH when the Federal agency maintains a registry and provides such information to NIH. Registration of non-NIH-funded research with the NIH Office of Recombinant DNA Activities (ORDA) is described in the APS. (The information required is similar to that in an MUA for NIH-supported research.)"

X. *Amendment of Section IV–F–3.* Section IV–F–3 could be amended to read as follows:

"IV–F–3. *Voluntary Registration and Certification.* Any institution that it not require to comply with the Guidelines may nevertheless register its intent to comply by submitting the appropriate information to ORDA. NIH will accept requests for certification of host-vector systems proposed by the institution. The submitter must agree to abide by the physical and biological containment standards of the NIH Guidelines."

9. *Proposed amendment of appendix E of the guidelines.* Dr. Fritz Reusser of the Upjohn Company, has requested that two sections of Appendix E be amended in order to include additional *Streptomyces* species. The proposed revised sections would read as follows:

"*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus* and *S. venezuelae* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions."

"*Streptomyces coelicolor, S. Aureofaciens, S. rimosus, S. griseus, S. cyaneus* and *S. Venezuelae* can be used as hosts for the cloning of DNA derived from *B. Subtilis, E. Coli,* K–12, or from *S. Aureus* vectors that have been approved for use in *B. subtilis,* under P2 conditions, using as vectors any plasmids indignous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

10. *Request for exemption under Section I–E–4.* Dr. Walter R. Guild of Duke University, has proposed that *Streptococcus sanguis* and *Streptococcus pneumoniae* be included under the exemption category of Section I–E–4, and added to Appendix A of the Guidelines on the basis that these bacteria exchange chromosomal genes in both directions by known physiological processes.

11. *Proposed amendments of Section IV–E–2.* Dr. Irving Johnson of Eli Lilly and Company, submitted a number of proposals for changes in the Guidelines for consideration at the June 5–6, 1980 RAC meeting. Some were deferred. He has now resubmitted two proposed revisions of the Guidelines.

A. Following the first sentence of the second paragraph of IV–E–2, the following sentence would be inserted:

"Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

[When this proposal was discussed at the June 1980 meeting, objections were raised to inclusion of the words "of industry." A motion passed to include a modification of this sentence in the Guidelines as follows:

> "Members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

This was followed, however, by passage of a motion to reconsider, and then passage of a motion to defer consideration until the September RAC meeting].

B. A new Section IV–E–2–b would be added to read as follows:

"A permanent subcommittee of the RAC shall be responsible for advising the Director, NIH, on the actions, listed in Section IV–E–1–b–(3)–(d) pertaining to large-scale applications. Submissions that are in compliance with the *Guidelines* may be recommended by the subcommittee to the Director of NIH for approval. The subcommittee shall also be authorized to consider preliminary plans for large-scale operations and to recommend approval contingent upon completion of the large-scale facility according to those plans. The subcommittee will be responsible for expeditiously processing applications."

12. *Request to include Vibrio cholerae in Appendix A.* Dr. John A. Mekalanos, of Harvard Medical School, has requested the NIH to consider the addition of *Vibrio cholerae* to Sublist A in Appendix A of the Guidelines. The organisms on this list are those that are exempt from the Guidelines on the basis that they have been shown to exchange genetic material by known physiological processes.

13. *Request for consideration of appropriate containment levels.* Drs. B. S. Montencourt and D. E. Eveleigh of Rutgers University, have requested permission to clone DNA derived from *Pseudomonas* strains that are non-pathogenic to plants or animals in an *E. coli* K-12 host and to be returned to *Zymomonas mobilis.*

14. *Proposed containment for cloning between members of the actinomycetes group.* Dr. Dean Taylor of the Smith Kline and French Laboratories, has proposed that the third entry in Appendix E of the Guidelines be modified to read:

"P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for those species which are known to be pathogenic for man, animals, or plants."

This proposal was made previously by the RAC Working Group on Prokaryotic Host-Vectors Other Than *E. coli* and appeared in the **Federal Register**, April 13, 1979 *44* (73): 22316. The RAC considered the proposal at its May 21–23, 1979 meeting and recommended to restrict this so that it did not include the entire *Actinomycetes* group but rather only the genera *Streptomyces* and *Micromonospora.* The Director, NIH, accepted this recommendation and the action was published in the **Federal Register**, July 20, 1979 *44* (141): 42916, and appears as the third entry in Appendix E of the Guidelines.

15. *Request to clone Schizosaccharomyces pombe* DNA in *Schizosaccharomyces pombe.* Dr. Benjamin Hall of the University of Washington, requests permission to clone *Schizosaccharomyces pombe DNA in Schizosaccharomyces pombe* using approved HVI *Saccharomyces/E. coli* hybrid plasmids as vectors. Dr. Hall requests that Pl be established as the appropriate level of physical containment. Dr. Hall points out that *Schizosaccharomyces pombe* has been the subject of intense genetic studies in the laboratory and traditionally has been used to ferment beverages for human consumption.

Additional Announcements of the Director, NIH

Section IV–E–1–b–(3)–(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Accordingly, several requests for authorization to culture, on a large-scale, recombinant DNA host-vector systems have been received and reviewed by the NIH.

1. *Genentech, Inc.* On the recommendation of the RAC, the following requests from Genentech, Inc., have been approved by the Director, NIH:

A. On July 22, 1980, the Director, NIH, approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 host-vector systems containing plasmids into which have been ligated a combination of chemically synthesized DNA and cloned cDNA coding for human growth hormone.

B. On July 22, 1980, the Director, NIH, approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 Host-vector systems containing plasmids into which have been ligated a combination of chemically synethesized DNA and cloned cDNA coding for human leukocyte or fibroblast interferon.

Both requests were approved with the understanding that Genentech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site, and to certain additional stipulations.

The principal investigators are Drs. Michael Ross and Norm S. C. Lin. The work is to be done, as stipulated by the Genentech, Inc. submission, at the P1–LS level of containment at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080.

2. *Burns-Biotec Laboratories, Inc.* On July 22, 1980, The Director, NIH, approved a request from Burns-Biotec Laboratories, Inc., a wholly-owned subsidiary of Schering Corporation, for large-scale culture of an EK1 host-vector system containing a plasmid coding for human leukocyte interferon in a 1000 liter fermentor (up to 750 liter working volume) at the P1–LS level of containment.

This request was approved with the understanding that Burns-Biotec Laboratories, Inc., has agreed to permit an observer, designed by NIH, to visit the facilities if NIH should choose to inspect the site. The principal investigator for this project is Dr. Donald E. Baldwin. The large-scale growth of the organisms is to be carried out at plant facilities located in Elkhorn, Nebraska.

OMB's "Manadatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8(b)(4) and (5) of that Circular.

Dated: August 14, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

SEPTEMBER 25-26, 1980

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

SEPTEMBER 25-26, 1980

The Recombinant DNA Advisory Committee (RAC) was convened for its twentieth meeting at 9 a.m. on September 25, 1980, in the Linden Room, Linden Hill Hotel, 5400 Pooks Hill Road, Bethesda, Maryland 20014. Mr. Ray Thornton (Chairman) President, Arkansas State University, presided. In accordance with Public Law 92-463 the meeting was open to the public, except for the review of proposals involving proprietary information as the last item of business on September 26, 1980.

Committee members present for all or part of the meeting were:

Dr. Abdul Karim Ahmed; Dr. Kenneth Berns; Dr. Winston Brill; Dr. Allan Campbell; Mrs. Zelma Cason; Dr. Nina Fedoroff; Dr. Richard Goldstein; Dr. Susan Gottesman; Dr. Jean Harris; Ms. Patricia King; Dr. Sheldon Krimsky; Dr. Werner Maas; Dr. James Mason; Dr. Gerard McGarrity; Dr. Elena Nightingale; Dr. Richard Novick; Dr. Ramon Pinon; Dr. John Scandalios; Dr. Luther Williams; and Dr. William J. Gartland, Jr., Executive Secretary.

A Committee roster is attached. (Attachment I)

The following ad hoc consultants to the Committee were present:

Dr. Vee J. Gill, Clinical Center, NIH; Dr. Vernon Knight, Texas Medical Center, Houston, Texas.

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[1] The RAC is advisory to the NIH, and its recommendations should not be considered as final and accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

The following non-voting members and liaison representatives were present:

Dr. Darryl Banks, Environmental Protection Agency; Dr. Charlotte Bell, U. S. Department of Justice; Dr. Howard Bermann (ALT.), U. S. Veterans Administration; Dr. Donald DeVincenzi, National Aeronautics and Space Administration; Dr. George Duda, U. S. Department of Energy; Dr. Timothy J. Henry, Food and Drug Administration; Dr. Herman Lewis, National Science Foundation; Dr. David Logan, U. S. Department of Labor; Dr. Sue Tolin, U. S. Department of Agriculture; and Dr. William J. Walsh, III, U. S. Department of State.

Other National Institutes of Health staff present were:

Dr. Marilyn Bach, NIAID; Dr. Stanley Barban, NIAID; Dr. W. Emmett Barkley, ORS; Mrs. Betty Butler, NIAID; Dr. John Irwin, ORS; Dr. Richard Krause, NIAID; Ms. Chris Krutzsch, NIAID; Dr. Robert McKinney, ORS; Dr. Elizabeth Milewski, NIAID; Dr. Stanley Nagle, NIAID; Dr. John Nutter, NIAID; Dr. Maxine Singer, NCI; Dr. Bernard Talbot, OD; Dr. Rudolf Wanner, ORS; and Dr. Burke Zimmerman, OD.

Others in attendance for all or part of the meeting were:

Dr. Lucile Adamson, Occupational Safety and Health Administration; Dr. E. A. Agostini, Pfizer, Inc.; Dr. D. E. Baldwin, Schering Corp.; Ms. Irene Brandt, Eli Lilly & Co.; Dr. Peter Bostock, New Brunswick Scientific Co., Inc.; Ms. Vicky Cahan, McGraw Hill; Dr. C. T. Chen, Occupational Safety and Health Administration; Mr. Jeffrey Christy, Blue Sheet; Mr. Chris Coley, Harvard Medical School student; Dr. Aileen Compton, Smith-Kline & French; Mr. David Dickson, Nature; Dr. Diana Dutton, Stanford University; Dr. John Grupenhoff, Washington Counsel/Medicine and Health; Dr. Lowell Harmison, Office of Assistant Secretary for Health; Dr. Zsolt Harsanyi, Office of Technology Assesment; Ms. Flo Hassell, Office of Assistant Secretary for Health; Mr. Phil Hilts, Washington Post; Dr. Paul Hung, Abbott Research Laboratories; Dr. James Hunt, Chemapec; Dr. Evelyn Hurlburt, Johns Hopkins School of Hygiene; Mr. Philip Janus, Office of Assistant Secretary for Health; Dr. Dorothy Jessup, U. S. Department of Agriculture; Dr. Irving Johnson, Eli Lilly & Co.; Dr. Attila I. Kadar, Food and Drug Administration; Mr. B. Khosrovi, Cetus Corp.; Mr. Edward Korwek, Kells & Heckman; Ms. Ann Lallande, McGraw Hill; Dr. Paul Leibowitz, Schering Corp.; Ms. Carter Leonard, Blue Sheet; Dr. M. A. Levin, Environmental Protection Agency; Dr. James McCullough, Library of Congress; Dr. Henry I. Miller, Food and Drug Administration; Mr. Claude Nash, Smith-Kline & French; Dr. DeLill Nasser, National Science Foundation; Dr Ann Norberg, Monsanto Co.; Dr. Arthur Norberg, National Science Foundation; Mr. Seth Pauker, National Institute for Occupational Safety and Health; Dr. William Pilacinski, Molecular Genetics, Inc.; Mr. Alvin Polan, SELF; Dr. John Richardson, Centers for Disease Control; Mr. Harold Schmeck, New York Times; Dr. Brian Sheehan, Consultant, Amos Corp.; Mr. Vincent Simmons, Genex Corp.; Dr. Mark Smith, CNAM, Paris; Mr. Jeff Swarz, Teknekron, Inc.; Mr. Dean Taylor, Smith-Kline & French; Dr. Charles Weiner, Massachusetts Institute of Technology; Dr. Marvin Weinstein, Schering Corp.; Dr. Susan Wright, University of Michigan; Dr. Bill Young, Genentech, Inc.; and Dr. Robert Zaugg, Teknekron, Inc.

I. CALL TO ORDER AND OPENING REMARKS

Mr. Thornton introduced a new RAC member, Dr. Nina Fedoroff, Carnegie Institution of Washington, and Dr. Vernon Knight, Baylor College of Medicine, Texas Medical Center, an ad hoc consultant.

II. MINUTES OF THE JUNE 5-6, 1980 MEETING

Ms. Cason said she found the draft minutes (tab 939) of the June 5-6, 1980 RAC meeting to be complete with no substantive errors. Ms. Cason moved approval of the minutes with suggested corrections of typographical errors. The minutes were unanimously accepted.

III. SURVEY OF INSTITUTIONAL BIOSAFETY COMMITTEES IN CALIFORNIA

Mr. Thornton invited Dr. Diana Dutton, Health Services Research, Stanford University School of Medicine, Stanford, California, to present the results (tab 937) of the survey of California Institutional Biosafety Committees (IBCs). Dr. Dutton said a group at Stanford University began about two years ago to study the process of policy making in biomedical innovation, particularly the public's role in the process. The group viewed the mandating of IBC composition by the 1978 National Institutes of Health (NIH) Guidelines as an experiment in local public participation in science regulation. She emphasized that the findings she would present to the RAC were suggestive but not definitive.

Dr. Dutton said the survey consisted of a questionnaire sent to all IBC chairpersons in California, and a separate questionnaire sent to all nonaffiliated members of California IBCs. Ninety-five percent of all IBC chairpersons responded (19 responses out of 20 surveyed) as did ninety-two percent of all non-affiliated members contacted (45 out of 49). Some of the findings were summarized in a document which Dr. Dutton distributed at the meeting (Attachment II).

The average IBC is composed of eleven members. The majority of committee members are scientists: twenty-eight percent are "recombinant DNA scientists," and twenty-seven percent are "other life scientists." Public health officials constitute twelve percent of the typical committee. Other categories of membership include students, laboratory workers, local citizens, etc.

The majority of IBC chairpersons (56%) indicated that the IBC had no relationship to local government. The average number of meetings held in 1979 was 4.3. On the average, sixty-three percent of the meetings were not regularly scheduled. Four of the nineteen committee chairpersons indicated that some meetings were held by telephone or in writing. The agenda of the typical meeting was roughly divided equally between review of MUAs and discussion of policy matters and other business. The committees on the average reviewed sixteen MUAs in 1979, but the range went from one MUA to sixty-eight MUAs. The amount of time spent in review of an MUA averaged thirty-two minutes, but values ranged from six minutes per MUA to an hour and forty-three minutes per MUA. Four percent of all MUAs were rejected. Dr. Maas pointed out that draft MUAs are often first checked informally with the IBC Chairman or with ORDA before formal submission to the IBC.

Less than a third of the committees felt that special health surveillance was necessary in regard to emergency plans in case of accidental spills. Some IBCs focused on internal laboratory procedure, others focused on the relationship between laboratories and external agencies. The task of training laboratory staff was delegated primarily to the principal investigator. Training for IBC members ranged from none to a fairly elaborate system involving training documents, discussions and laboratory tours.

The assessment of the Guidelines and IBC function by the chairpersons and the nonaffiliated members was, on the whole, positive. The chairpersons tended to emphasize the positive aspects of Guideline flexibility, while the nonaffiliated members stressed the watchdog and public relations role of the IBCs.

While the majority of non-affiliated members are local citizens or public health officials, some twenty-five percent are life or recombinant DNA scientists. Dr. Dutton felt that this is not in technical violation of the Guidelines but is in some conflict with the intent of the Guidelines. Both the chairpersons and the nonaffiliated members were modestly positive about the role of nonaffiliated members. Sixty-one percent of the chairpersons felt the stipulation that twenty percent of the IBC membership be nonaffiliated was appropriate. Thirteen percent of nonaffiliated members thought that their contributions were not valuable. Approximately half of the committees said all of their meetings were open to the public; about half said none of their meetings were open to the public. Dr. Dutton felt IBCs were not making sufficient effort to reach out into the community and solicit participation. In conclusion, Dr. Dutton noted great diversity in California's IBCs with respect to their membership, scope of activities, performance, etc.

The Committee discussed with Dr. Dutton her data and her interpretation of them. Dr. McGarrity questioned whether unannounced meetings could be termed open public meetings. Dr. Berns said that advertising a meeting far in advance introduced an element of inflexibility into scheduling.

Dr. Ahmed asked Dr. Dutton to state her conclusions on industrial IBCs. Dr. Dutton replied that industrial IBCs compared to university IBCs are less accessible to the public, have higher MUA approval rates, have more elaborate health surveillance programs, and provide better training for staff and IBC members.

IV. MEETING OF INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) CHAIRPERSONS AND IBC SURVEY

Dr. Krause, the Director of the National Institute of Allergy and Infectious Diseases (NIAID), said that the NIH sponsored an IBC chairperson meeting approximately two years ago. He said NIAID now would sponsor a second IBC chairperson meeting on November 24-25, 1980. Dr. Krimsky and several IBC Chairmen are on the Planning Committee. Dr. Nutter said the November 1980 meeting would serve two purposes: (1) it will permit IBC chairpersons to meet and attempt to resolve common problems, and (2) it is viewed as the first stage in a formal evaluation process. He invited RAC members to attend. He then reviewed the format of the meeting; a plenary introductory session the first morning, will be followed by three separate workshops that afternoon. The workshops deal with (1) the IBC as a means of implementing institutional oversight, (2) health surveillance, monitoring and certification, and (3) procedures and operations. A second plenary session dealing with workshop reports is planned for the second morning. This session will be followed by a plenary session dealing with other federal regulations and guidelines impacting on biomedical science.

Dr. Nutter noted that a Request for Proposals (RFP) is being developed to evaluate all IBCs. Based on the results of the November 24-25 meeting, the RFP may be reviewed. Dr. Gottesman noted that Dr. Dutton's survey evaluated public participation in an IBC operation. She said a second important question is the effectiveness of the IBC in assuring compliance with the containment levels of the Guidelines.

V. PROPOSED PROCEDURES FOR REVIEW OF LARGE-SCALE APPLICATIONS

Dr. Logan of OSHA distributed copies of a September 24, 1980 letter from Dr. Bingham to Dr. Fredrickson (Attachment III). Dr. Gottesman reviewed the background of the proposal for revised procedures for review of large-scale applications. She noted that at the past few RAC meetings, there has been extensive discussion of the role of RAC and of the NIH in the review of large-scale proposals particularly submitted by industrial concerns. She moved approval of the following proposal which had been published in the Federal Register (tab 931/7) of August 21, 1980 for comment:

> "The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. The RAC will determine if a given recombinant DNA-containing strain is rigorously characterized and the absence of harmful sequences established. Such a determination shall include specification of a containment level (P-LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industrial scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC."

Following this proposal, there appear in the Federal Register of August 21, 1980, proposed revised application procedures to implement this change. Dr. Gottesman said under this proposal the IBC would accept responsibility for assuring adherence to the physical containment guidelines. She said RAC would continue to evaluate the biology of the recombinant clones and the host-vector systems, but no longer deal with prior review of physical containment in individual applications.

Dr. Krimsky said he had supported this proposal at the June 1980 RAC meeting. He had felt then that a government agency, such as the Occupational Safety and Health Administration (OSHA), might more appropriately perform the function. He said that he had since learned that RAC's prior review activity is unique; there is no OSHA mandate for such prior review. He felt the RAC's prior review process serves an important function. No other agency would perform this prior review should RAC extricate itself from the process. He proposed, as an alternative proposal, that there be established a subcommittee of the RAC made up of some members of the RAC and some members of NIH staff with expertise in facilities and technologies, and that the subcommittee request representation from the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), the Center for Disease Control (CDC), and the Environmental Protection Agency (EPA). In this proposal, the subcommittee would review engineering and technology, and its review would be transmitted directly to the Director, NIH.

Dr. Goldstein seconded Dr. Krimsky's proposal. Dr. Gottesman did not accept the alternative proposal. Dr. Krimsky agreed to withdraw his motion with the understanding that it would be reconsidered later in the meeting. Ms. King stated that Dr. Gottesman's proposal is an acceptable compromise, although she would prefer that the RAC withdraw from all review of industrial proposals. Dr. Mason noted that the RAC doesn't monitor even small-scale experiments. Dr. Gottesman said that the crux of the issue is whether evaluation of individual physical containment facilities by RAC is appropriate; she said it is not. Dr. Berns said that the RAC should consider only the biology of the systems. Dr. Goldstein supported Dr. Krimsky's proposal; he said that there is not enough information available on local IBCs.

Dr. Logan of OSHA felt RAC prior review of industrial applications serves an important function. He said that potential problems have been identified by RAC, and he hoped the RAC would consider Dr. Krimsky's proposal.

It was suggested that Dr. Gottesman's proposal could be divided and the last sentence of the motion voted on separately. Dr. Gottesman did not agree. Dr. Williams supported Dr. Gottesman's position saying he preferred to vote on the entire proposal. Ms. King agreed.

Mr. Thornton called the question on Dr. Gottesman's motion. The RAC accepted the language as published in the Federal Register (931/7) by a vote of twelve in favor, five opposed, and one abstention. Dr. Goldstein requested that his vote of opposed be recorded. Dr. Ahmed also requested that his vote of opposed be recorded saying he is opposed to a voluntary compliance scheme.

VI. PROPOSED AMENDMENT OF SECTION IV-E-2

Ms. Cason introduced the proposal (tabs 918, 931/11/A) from Dr. Irving Johnson, Eli Lilly and Company, to amend Section IV-E-2 of the Guidelines. Ms. Cason recounted the history of the proposal. She noted that several proposals to amend the Guidelines were submitted by Dr. Johnson for evaluation at the June 5-6, 1980 RAC meeting. Consideration of some of these proposals had been deferred. She said Dr. Johnson has resubmitted two of those deferred proposals for consideration at the September 6-7, 1980 meeting. One proposal would insert the following language after the first sentence of the second paragraph of IV-E-2:

> "Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

Ms. Cason said that when this proposal was discussed at the June 1980 meeting, objections were raised to inclusion of the words "of industry." A motion recommending the following modified language was passed at the June 1980 meeting:

> "Members should be chosen to provide expertise in fermentation technology engineering and other aspects of large-scale production."

She said further discussion of the modified language had been deferred to the September 1980 meeting. Ms. Cason said the appointment of a member with fermentation technology expertise would afford RAC an additional dimension, and moved approval of the modified language.

Dr. Krimsky asked whether Ms. Cason's proposal might more appropriately be evaluated after his proposal to form a subcommittee has been discussed. Dr. Gottesman replied that RAC has developed large-scale guidelines and would continue to review large-scale applications. Fermentation technology expertise would be desirable whether or not a subcommittee as proposed by Dr. Krimsky is established.

Dr. Johnson said he had inserted the words "representatives of industry" in his proposal as most expertise in this area is in industry or in academia consulting for industry. Dr. Campbell said he did not regard himself as a "representative" of academic biology but as an individual with certain expertise. He said he objected to the language of Dr. Johnson's proposal.

By a vote of thirteen in favor, two opposed, and two abstentions the RAC recommended the language proposed by Ms. Cason, i.e.:

> "Members should be chosen to provide expertise in fermentation technology engineering and other aspects of large-scale production."

VII. PROPOSED BIOSAFETY GUIDELINES FOR BIOMEDICAL LABORATORIES

Dr. John Richardson, CDC, said the proposed guidelines (tab 915) are a more comprehensive listing of microorganisms than the 1974 CDC Classification of Etiologic Agents on the Basis of Hazard. He said the proposed biosafety guidelines designate four biosafety levels which correspond closely to the P-levels of the NIH Guidelines for Research Involving Recombinant DNA Molecules. The CDC guidelines propose three categories of laboratory activities (1) manipulation of small quantities or low concentrations of the agent, (2) manipulation of large quantities or high concentrations of the agent, and (3) manipulation of vertebrate animals infected with the agent. Ten thousand copies of the proposed biosafety guidelines will be distributed for comment.

Dr. Berns asked Dr. Richardson to comment on the status of the CDC guidelines. Dr. Richardson replied that the CDC guidelines are voluntary. He said that the Department of Health and Human Services (HHS) does not have the authority to impose such guidelines as on an intrastate basis.

VIII. PROPOSED PROCEDURES FOR REVIEW OF LARGE-SCALE APPLICATIONS (Continued)

Dr. Krimsky returned to the concept which had been discussed previously (Section V) of a new RAC Subcommittee to deal with large-scale proposals. He would have the Subcommittee membership include RAC members as well as members from NIH and other agencies who have expertise in large-scale fermentation technology. He suggested it be indicated that approval for facilities does not in any way suggest that there is an enforcement operation.

The question was posed whether the proposed subcommittee would review the physical containment aspects of individual large-scale applications after RAC has evaluated biological containment. Dr. Krimsky said it would; the RAC would review the biology of the systems, the subcommittee would subsequently review the physical containment, surveillance, monitoring, etc. It was suggested that a straw vote be taken on the concept. By a straw vote of eight in favor to three opposed, the RAC supported the concept, and it was agreed that more precise language would be developed for further consideration. (Discussion of this proposal continues in Section XVI).

IX. PROPOSED CHANGES IN REGISTRATION REQUIREMENTS

Dr. Maxine Singer introduced her proposal (tabs 917, 931/8, 934, 935, 938, 942-948, 952) to amend administrative requirements of the Guidelines. Dr. Singer said her proposal was intended to (1) eliminate the requirement for central registration of recombinant DNA projects at NIH, and (2) disengage the review of recombinant DNA proposals from the grant review process. She felt that safety is primarily maintained in the laboratory and reviewers close to the experimental locale render more meaningful review in terms of safety. She added that Genetic Manipulation Advisory Group (GMAG) in the United Kingdom has transferred the responsibility for categorization of most experiments to the local biosafety committees (tab 950). Dr. Wright said that the GMAG situation and the NIH situation are not analogous. She pointed out that GMAG clearly defines the composition of the biosafety committee and that British laboratories are regulated and inspected by the government, unlike laboratories in the United States. Dr. Singer agreed.

Dr. Harris said that she believed that the NIH is approaching a point in experience and history where Dr. Singer's proposal would be reasonable. She noted that the issue revolves around RAC perception of and comfort with the sophistication and rigor with which IBCs pursue review. In light of the upcoming review of IBC performance she recommended delay until the IBC survey is completed.

Dr. Gottesman suggested that Dr. Singer's proposal could be divided into two parts; i.e., the elimination of registration with NIH, and the elimination of IBC prior review. Dr. Mason said he would feel comfortable eliminating NIH registration, but opposed allowing the investigator to bypass the IBC. Drs. Harris and Williams agreed with Dr. Mason. Dr. Goldstein expressed reservation. Dr. Goldstein noted that IBC efficiency has not yet been evaluated. Dr. Krimsky concurred saying that evidence for evaluating IBC efficiency is, at the moment, very sparse. He questioned whether elimination of ORDA review will increase or decrease an IBC's sense of responsibility.

Dr. Singer said that it is not the intent of her proposal to eliminate IBC review of registration documents. It would, however, allow the investigator to begin the experiment upon submission of the registration documents to the IBC, without waiting for IBC review. Dr. Goldstein expressed reservations; he noted that at the present time IBC effectiveness has not been fully evaluated. Dr. Fedoroff and Ms. King said they believed that IBCs are functioning well. Dr. Berns said he is aware of specific instances in which the investigator and the IBC disagreed on interpretation of the Guidelines. He opposed the elimination of IBC prior review.

Dr. Gottesman then moved the following three part proposal:

(1) Eliminate the requirement for NIH review of IBC decisions on any experiments for which containment levels are specified in the Guidelines,

(2) Defer consideration of eliminating prior review of experiments (by the IBC) until the frequency of principal investigator error in selecting the appropriate containment levels has been determined, and

(3) IBCs keep records of recombinant DNA research done in the institution, including a record of the frequency of errors in classification of experiments by the principal investigator.

Ms. King seconded the motion.

Dr. Novick proposed that ORDA continue to receive, collect and evaluate MUAs during the interim period in which the IBC survey is being conducted. Dr. Gottesman did not accept this amendment to her proposal. Dr. Mason suggested that IBC function could be monitored without central registration.

Mr. Coley asked whether Dr. Singer's proposal might shut off lines of communication between local IBCs and ORDA. He noted some uncertainty at the IBC level in interpretation of the Guidelines. Dr. Singer replied that her proposal would not alter ORDA's advisory function to IBCs and principal investigators (PIs).

Dr. Campbell noted that the NIH Guidelines set minimal standards and that institutions, if they wish, may impose additional requirements beyond what the Guidelines require.

Dr. Fedoroff asked Dr. Gottesman why IBC review should be required, rather than letting the PI interpret the Guidelines. Dr. Gottesman replied that IBC prior review should continue for the following reasons: (1) IBC prior review would result in a more thorough review by individuals with varied perspectives; (2) The IBC has less of a conflict of interest in setting containment levels; (3) The IBC is more practiced in reading the Guidelines and evaluating proposals; and (4) RAC has some data suggesting that the IBCs function well, but no data on how correctly PIs evaluate containment levels.

The RAC then voted separately on the different parts of the motion.

The RAC voted fifteen in favor, three opposed, with no abstentions, to eliminate the requirement for NIH review of IBC decisions on any experiments for which containment levels are specified in the Guidelines. Dr. Goldstein wished to be recorded as voting against the proposal.

The RAC voted twelve in favor, five opposed, with one abstention, to defer consideration of eliminating prior review of experiments by the IBCs until the frequency of principal investigator error in selecting the appropriate containment levels has been determined.

The RAC voted seventeen in favor, none opposed, with one abstention, that IBCs maintain records of recombinant DNA research done in their institution, including a record of the frequency of errors in classification of experiments by the principal investigator. Dr. Krimsky wished to be recorded as abstaining from the vote.

Dr. Singer pointed out that the language of Dr. Gottesman's proposal did not eliminate the requirement for registration with NIH. Dr. Gottesman moved a fourth provision, as follows:

> "the IBCs no longer need register with NIH, recombinant DNA experiments for which containment levels are specified in the Guidelines"

Dr. Ahmed proposed to amend Dr. Gottesman's proposal so that NIH be required to collect, on a periodic basis, information from IBCs regarding all recombinant DNA research being conducted, and that this information be made available for public inspection. Dr. Gottesman did not accept Dr. Ahmed's amendment. Dr. Ahmed agreed to reoffer his amendment following a vote on Dr. Gottesman's motion. The RAC voted fifteen in favor, three opposed, with no abstentions that the IBC no longer need register with NIH, recombinant DNA experiments for which containment levels are specified in the Guidelines. Dr. Goldstein wished to be recorded as voting against the motion.

Dr. Ahmed then moved that there should be an annual summary report by the IBC to NIH on all recombinant DNA research at the institution. Dr. Novick did not consider it necessary to report exempt experiments. Dr. Ahmed wished to include all recombinant DNA experiments. The RAC voted against the motion by a vote of three in favor, and thirteen opposed.

X. UPDATE OF NIH PROGRAM TO ASSESS THE RISKS OF RECOMBINANT DNA RESEARCH

Dr. Krause directed the committee to tab 933, the proposed first annual update of the NIH Program to Assess the Risks of Recombinant DNA Research. Dr. Krause reviewed the history of the program. The Secretary of Health, Education and Welfare (HEW), [now named Health and Human Services (HHS)] requested that the NIH prepare a risk assessment plan, that RAC review it, that it be published in the Federal Register for comment, made final, and that it be updated annually.

Dr. Krause said the Risk Assessment Program collects and analyzes data on potential hazards of recombinant DNA organisms. Dr. Krause reviewed progress on the issues developed at the Pasadena, California, Risk Assessment

Meeting held in April 1980. He said a Request for Proposals (RFP), to determine if mice can mount an antibody response to insulin produced by E. coli host-vector systems, will be presented to the NIAID Advisory Council in the near future. In addition, a Request for Grant Applications (RFA) developed in conjunction with the National Institute of Arthritis, Metabolism and Digestive Diseases to investigate the absorption of peptide hormones by the intestine will soon be presented to the NIAID Advisory Council.

Dr. Krause reported that a contract has been awarded to the University of Minnesota to develop a comprehensive course on microbiological principles and techniques for work with potentially hazardous agents, including recombinant DNA organisms.

Dr. Krimsky asked if NIAID might prepare a review article on risk assessment on recombinant DNA. Dr. Krause said he would take this suggestion under advisement. Dr. Nutter noted that the annual update synopsizes information on the progress made during the year. Dr. Goldstein supported Dr. Krimsky's position saying that such a review would be valuable. Dr. Williams pointed out that the update contains a summary and that full reports had been published in the Recombinant DNA Technical Bulletin and in refereed journals. Dr. Wright requested that the update be footnoted and the sources cited. Dr. Krause invited individual comments from all RAC members during the 90 day public comment period.

XI. PROPOSED HV2 BACILLUS SUBTILIS HOST-VECTOR SYSTEMS

Dr. Campbell noted that the request (tabs 931/4, 936) from Dr. William F. Burke, Jr., Arizona State University, to certify Bacillus subtilis strain ASB298 as the host component of an HV2 host-vector system had been discussed at the June 1980 meeting. He said the RAC had requested additional data on DNA transfer by transformation from ASB298 to other Bacilli. Dr. Burke provided supplemental information demonstrating: (1) in soil, plasmid pBD64 does not transform competent B. subtilis under conditions where chromosomal DNA transforms 40% of competent recipient cells; (2) plasmid-bearing wild-type B. subtilis donates a barely detectable number of plasmids to competent recipient bacteria in the soil, whereas no transfer from ASB298 was detected; and (3) under optimal laboratory conditions, ASB298 donates a chromosomal marker at barely detectable levels.

Dr. Campbell questioned whether plasmid transformation frequency had been measured under optimum conditions, as the recipient bacteria did not contain homologous plasmids. Dr. Gottesman moved that ASB298 be certified as the host component of an HV2 system on the basis of (1) the poor survivability of the strain; (2) its poor ability to donate DNA for transformation; (3) the high probability that plasmid vectors will transform very poorly.

The vector components would be the plasmid vectors already approved for HV1 Bacillus subtilis systems. The motion was adopted by a vote of fourteen in favor, none opposed, and two abstentions.

XII. PROPOSED REVISION OF SUBSECTIONS OF SECTION III-C-1-e

Dr. Berns presented the history of the proposal (tabs 920, 931/6) to revise Section III-C-1-e-(1)-(b) of the Guidelines. A notice appeared in the Federal Register of January 31, 1980, concerning proposed revision of Section III-C-1-e, and its subsections. It was recommended that Sections III-C-1-e, III-C-1-e-(1), III-C-1-e-(1)-(a), and III-C-1-e-(1)-(b), of the Guidelines be changed and that a new Section III-C-1-e-(1)-(c) be added. Section III-C-1-e-(2) would remain unchanged. The RAC, at its March 6-7, 1980 meeting, recommended adoption of Sections III-C-1-e, III-C-1-e-(1), and III-C-e-(1)-(a) as published in the Federal Register of January 31, 1980 with certain modifications in Section III-C-1-e-(1)-(a). The Director, NIH, accepted this recommendation and promulgated the following sections in the Federal Register of April 14, 1980:

> "III-C-1-e. All Viral Vectors.
>
> III-C-1-e-(1). Other experiments involving eukaryotic virus vectors can be done as follows:
>
> "III-C-1-e-(1)-(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

At the March 1980 meeting, the RAC deferred consideration of Sections III-C-1-e-(1)-(b) and III-C-1-e-(1)-(c) pending accumulation of additional data. A working group met on May 13, 1980 in Miami Beach, Florida, to discuss appropriate containment for experiments in which less than two-thirds of a eukaryotic viral genome is rescued with helper virus. The language developed by the working group appeared in the Federal Register of August 21, 1980:

> "III-C-1-e-(1)-(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with a helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents (1)."

Dr. Berns moved acceptance of the language in the Federal Register.

Dr. Gottesman asked if the levels specified in the working group proposal were higher or lower than the case-by-case assignments which have been made to date under the current Section III-C-1-e-(1)-(b). Dr. Barban replied that in the use of SV40 in some rescue experiments, containment is currently P3, and would, if this proposal were adopted, be P2.

Dr. Novick asked Dr. Berns to review the issues. Dr. Berns said the major consideration is whether splicing of various viral genomes might produce a pathogen more dangerous than the parent viruses themselves. The working group consensus was that no indication suggested this possibility. Dr. Berns said viruses are the product of millions of years of evolution, and are selected for optimal function.

By a vote of ten in favor, five opposed and one abstention, the RAC recommended the revision of Section III-C-1-e-(1)-(b) of the Guidelines as published in the Federal Register on August 21, 1980.

XIII. REQUEST TO INCLUDE VIBRIO CHOLERAE IN APPENDIX A

Dr. Gottesman presented the request (tabs 909, 931/12) from Dr. John A. Mekalanos, Harvard Medical School, to add Vibrio cholerae to sublist A, Appendix A of the Guidelines. She said Dr. Mekalanos has presented data showing evidence of R factor transfer. Dr. Gottesman said that recombinant DNA experiments between organisms within each sublist of Appendix A are exempt from the Guidelines under Section I-E-4. Section I-E-4 exempts "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes..."

Dr. Gottesman said a discussion has continued concerning the criteria required to qualify organisms for inclusion in Appendix A. She said the issue revolved on whether R factor transfer was sufficient or whether evidence of chromosomal exchange should be required. Committee members have previously argued that where R factor exchange occurs, chromosomal exchange, if looked for assiduously, probably would be found. She said she has differed with the committee on this interpretation, and that her inclination would be to require evidence of chromosomal transfer.

Dr. Novick expressed some concern over the pathogenic nature of Vibrio cholerae but qualified his statement with the following points: (1) a virulent E. coli producing cholera toxin probably would not be worse than a virulent E. coli producing E. coli enterotoxin; and (2) cholera toxin is processed in V. cholerae; E. coli may not be able to produce an active toxin. He suggested that certain experiments might be permitted under P1 + EK1 containment conditions, but that V. cholerae should not be added to Sublist A of Appendix A. Dr. Mason concurred.

Dr. Campbell said he accepted R-factor exchange as evidence that chromosomal exchange is occuring and moved approval of Dr. Mekalanos' request. He said that the toxin question is covered by prohibition I-D-2. Dr. Mason questioned whether the RAC could vote on this issue in the absence of a definition of a "potent" toxin. Dr. Novick moved that Vibrio cholerae be added to Sublist A, Appendix A, but that deliberate cloning of toxin genes be excluded. Dr. Campbell accepted the amendment. The vote was five in favor, four opposed, and five abstentions.

Mr. Thornton suggested that the issue be reconsidered as the sentiment of the committee was clearly divided. By a vote of eleven in favor, one opposed, the RAC agreed to reconsider the previous vote. Dr. Mason then moved to defer action on the proposal until additional information is available on the experiments being proposed. By a vote of thirteen in favor, one opposed, the RAC deferred action on Dr. Mekalanos' proposal.

XIV. PROPOSED AMENDMENT OF APPENDIX E OF THE GUIDELINES

Dr. Campbell said that Dr. Fritz Reusser, The Upjohn Company, requested (tabs 916, 931/9) that two entries under Appendix E be amended to permit specified experiments be performed with additional Streptomyces species. Dr. Campbell suggested that each of the proposals be considered separately. The first proposal is as follows:

> "Bacillus subtilis strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from E. coli K-12 and Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae using NIH-approved Staphylococcus aureus plasmids as vectors under P2 conditions."

Dr. Campbell, recommended approval of the proposal. By a vote of fourteen in favor, none opposed, and three abstentions, the RAC adopted this proposal.

Dr. Reusser's second proposal is as follows:

> "Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus and S. venezuelae can be used as hosts for the cloning of DNA derived from B. subtilis, E. coli K-12, or from S. aureus vectors that have been approved for use in B. subtilis, under P2 conditions, using as vectors any plasmids indigenous to these Streptomyces species or able to replicate in these hosts by natural biological mechanisms."

Dr. Novick proposed that there should be a restriction limiting the type of plasmid vector allowed to be used. He proposed to amend the language to require use of "nonconjugative" plasmid vectors. Dr. Campbell accepted the amendment.

By a vote of fifteen in favor, none opposed, and two abstentions the RAC recommended the following language:

> "Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae can be used as hosts for the cloning of DNA derived from B. subtilis, E. coli K-12 or from S. aureus vectors that have been approved for use in B. subtilis, under P2 conditions, using as vectors any nonconjugative plasmids indigenous to these Streptomyces species or able to replicate in these hosts by natural biological mechanisms."

Dr. Novick asked if this modified language might restrict researchers already working under Appendix E. Dr. Taylor of Smith Kline and French Company replied that several investigators are developing conjugative plasmid vectors under this section of Appendix E. Dr. Novick suggested that more concrete language on this issue might be considered at the next meeting.

(Executive Secretary's note: The Director, NIH, in his decision deleted the requirement for use of "nonconjugative plasmids." The reasons for this decision are presented in the Federal Register of November 21, 1980).

XV. REQUEST FOR EXEMPTION OF STREPTOCOCCUS SANGUIS AND STREPTOCOCCUS PNEUMONIAE UNDER SECTION I-E-4

Dr. Gottesman presented the proposal (tabs 919, 927, 931/10) from Dr. Walter R. Guild, Duke University. Dr. Guild proposed that Streptococcus sanguis and Streptococcus pneumoniae be added to Appendix A of the Guidelines. Dr. Gottesman said the evidence for natural exchange of genetic material between the two organisms in both directions is good. She moved that these organisms be added to Appendix A as a new sublist.

By a vote of sixteen in favor, none opposed, and one abstention, the committee adopted the proposal.

XVI. PROPOSED PROCEDURES FOR REVIEW OF LARGE-SCALE APPLICATIONS (Continued)

Mr. Thornton reminded the committee that it had earlier (Section V) adopted language defining the RAC's role in the review of large-scale proposals and that Dr. Krimsky (Section VIII) had proposed a new RAC subcommittee be involved in the review.

Dr. Krimsky moved the following language:

> "An industrial review subcommittee of the RAC shall be established with the responsibility for advising the Director

> on procedures and facilities design pertaining to applications for large-scale operations."

> "After the full RAC has reviewed the biological containment requirements for a large-scale proposal, the subcommittee shall examine the applicant's plans for large-scale operations and issue recommendations to the Director on plant design, health surveillance, and environmental monitoring. The Director shall advise firms of recommended design parameters and operational procedures. The determination shall not be construed as NIH certification of industrial operations."

> "The subcommittee shall invite participation from NIH's biosafety staff, OSHA, NIOSH, CDC, Food and Drug Administration (FDA), EPA and the U. S. Department of Agriculture (USDA)."

Dr. Talbot pointed out that; (1) ad hoc "working groups" on specific issues can be established by the RAC Chairman; "subcommittees" can be established only by modification of the Committee charter, which requires approval of the Secretary of Health and Human Services.

Dr. Fedoroff expressed concern about the delays if first the full RAC, and then subsequently a subcommittee, were to be involved in the review process. Dr. Goldstein favored Dr. Krimsky's proposal as it would allow NIOSH, OSHA, etc., to participate more intimately in the decision making process. Dr. Gottesman said that she was opposed to continuation of review of equipment design in individual applications by either the full RAC or a subcommittee.

Dr. McGarrity suggested that the American Society for Microbiology (ASM), might develop specifications for large-scale fermentors and auxiliary equipment to minimize personnel exposure and biohazards as has been done with safety specifications of laminar flow biological safety cabinets.

Dr. Johnson of Eli Lilly said that Dr. Krimsky's proposal contradicted the proposal passed by the RAC earlier (Section V). He expressed a belief that industrial large-scale growth of microorganisms affords higher levels of containment than procedures in university laboratories. Dr. Simon of Genex Corporation suggested that Dr. Krimsky substitute the word "institutions" for the word "firms" in his proposal. Dr. Krimsky agreed.

Dr. Mason suggested that the proposed subcommittee would have to advise the full RAC on each recommendation; if the subcommittee is empowered with final authority without reporting to the full RAC, he would not consider it to be a subcommittee of the RAC. A procedure involving a report to the full RAC would entail considerable delay. He said the RAC should either (1) accept responsibility for full RAC review of physical facilities or (2) delegate that authority to the IBC as voted by the RAC yesterday, the option he preferred.

Dr. Logan of OSHA opined that prior review of physical facilities serves an important function. He said the legal counsel of the Department of Labor (DOL) believes that OSHA does not have the authority to conduct such prior review. He further stated that the Federal Interagency Advisory Committee on Recombinant DNA has no mandate to conduct this type of prior review. He said that if the RAC does not accept Dr. Krimsky's proposal, large-scale prior review of physical facilities will not occur. Dr. Berns asked if representatives of the various federal agencies would be able to legally vote in the proposed subcommittee. Dr. Logan said the legal counsel of the Department of Labor would have to determine the propriety of OSHA participation. Dr. Gottesman reminded the Committee that under the proposal previously voted, the RAC will continue to review biological aspects of applications. Dr. Ahmed said that while he personally did not wish to participate in the review of confidential material, he thought the prior review system was valuable. He supported Dr. Krimsky's proposal.

Dr. McGarrity asked how other federal agencies view recombinant DNA experiments and products. Dr. Miller of FDA said it is likely there will be some linkage between the FDA product approval process and compliance with other federal guidelines, including the NIH Guidelines for Research Involving Recombinant DNA Molecules. Dr. Logan said that NIOSH and OSHA were working closely to develop a plan for possible regulation, or at least procedures to protect safety and health.

Dr. Campbell said that the RAC has certain responsibilities. One such responsibility is to set and to revise standards, e.g., what constitutes P1-LS. A second responsibility is to monitor the functioning of the system. He said he would support Dr. Krimsky's proposal if the subcommittee was constituted to gather information and to advise RAC on large-scale technology, but not to perform review of individual projects.

Dr. Gottesman moved that the motion be amended to read as follows:

> "An industrial review subcommittee of the RAC shall be established with the responsibility for advising the full RAC on procedures and facilities design pertaining to applications for large-scale operations. The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, EPA, and USDA."

The subcommittee would not review individual proposals, but would review standards. Dr. Berns suggested that Dr. Gottesman's proposed language be amended to change the name of the subcommittee to the "Large-Scale Review Subcommittee." Dr. Gottesman accepted this amendment. Mr. Thornton asked Dr. Krimsky if he would accept Dr. Gottesman's amended language as an amendment to his original motion. Dr. Krimsky declined. Instead, Dr. Krimsky moved to amend Dr. Gottesman's amendment to read as follows:

> "A large-scale review subcommittee of the RAC shall be established with the responsibility for advising the entire RAC on large-scale standards and the Director of NIH on procedures and facilities design for large-scale operations."
>
> "After the full RAC has reviewed the biological containment requirements for a large-scale proposal, the subcommittee shall examine the applicant's plans for large-scale operations and issue recommendations to the Director on plant design, health surveillance, and environmental monitoring. The Director shall advise institutions of recommended design parameters and operational procedures. The determination shall not be construed as NIH certification of industrial operations."
>
> "The subcommittee shall invite participation from NIH's Biosafety Division, OSHA, NIOSH, CDC, FDA, EPA and USDA."

Dr. Krimsky said that it is the intent of his motion that the subcommittee will deal with prior review of individual applications. Dr. Federoff said that Dr. Krimsky's proposed language would introduce delays in the procedure.

Mr. Thornton called the question on Dr. Krimsky's proposed amended language. By a vote of three in favor, fifteen opposed, the RAC opposed Dr. Krimsky's proposed amended language.

Mr. Thornton then returned to Dr. Gottesman's proposed amended language. It was suggested that the words "...pertaining to large-scale operations" be substituted for the words "...pertaining to applications for large-scale operations" in the first paragraph of the motion. Dr. Gottesman agreed. It was reiterated that the subcommittee would not perform prior review of individual applications.

Mr. Thornton then called the vote on Dr. Gottesman's amendment to Dr. Krimsky's original motion. By a vote of thirteen in favor, four opposed, and one abstention, the RAC agreed to the amendment.

Dr. Campbell offered an amendment to add the words "and on the performance of local IBCs in reviewing physical containment facilities." The amended motion would read as follows:

> "A large-scale review subcommittee of the RAC shall be established with responsibility for advising the RAC on procedures and facilities design pertaining to large-scale operations, and on the performance of local IBCs in reviewing physical containment facilities. The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA and USDA."

Dr. Campbell explained that this provision would permit a review of how well IBCs are performing this function. Dr. Gottesman noted NIAID is planning to evaluate IBC function. She questioned whether a special effort was required in large-scale applications. Dr. Campbell said that the proposed subcommittee would have the expertise to evaluate this aspect of IBC function. By a vote of thirteen in favor, three opposed, and two abstentions, the RAC accepted the Campbell amendment.

The RAC then voted fifteen in favor, two opposed, and two abstentions to adopt the following amended proposal:

> "A large-scale review subcommittee of the RAC shall be established with responsibility for advising the RAC on procedures and facilities design pertaining to large-scale operations, and on the performance of local IBCs in reviewing physical containment facilities. The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA and USDA."

XVII. PROPOSED CONTAINMENT FOR CLONING AMONG MEMBERS OF THE ACTINOMYCETES GROUP

Dr. Brill introduced the request (tabs 925, 926, 931/14) from Dr. Dean Taylor, Smith, Kline and French Laboratories. Dr. Taylor proposed that the third entry in Appendix E of the Guidelines be modified to read:

> "P2 physical containment shall be used for DNA recombinants produced between members of the Actinomycetes group except for those species which are known to be pathogenic for man, animals or plants."

This proposal was made previously by the RAC Working Group on Prokaryotic Host-Vectors other than E. coli and appeared in the Federal Register, April 13, 1979, 44 (73): 22316. The RAC considered the proposal at its May 21-23, 1979 meeting and recommended to restrict this so that it did not include the entire Actinomycetes group but rather only the genera Streptomyces and Micromonospora. The Director, NIH, accepted this recommendation and the action was published in the Federal Register, July 20, 1979, 44 (141): 42916, and appears as the third entry in Appendix E of the Guidelines.

Dr. Brill noted that currently the Guidelines permit P2 containment to be used for DNA recombinants produced between Streptomyces and Micromonospora. Experiments using other genera of the Actinomycetes may be performed under P3 containment. He said there are few pathogenic organisms in the Actinomycetes genera and most of those organisms are pathogenic only in compromised hosts. He moved approval of the proposal. Dr. Scandalios concurred.

Dr. Taylor said pathogenic Actinomycetes are marginal pathogens; the hosts are generally compromised.

Mr. Thornton introduced Dr. Vee J. Gill of the Department of Clinical Pathology, NIH. Dr. Gill said that many genera of the Actinomycetes are not pathogenic for man, animals or plants.

Drs. Gill and Taylor said that experiments to demonstrate exchange among Actinomycetes have not been performed to any great extent. Dr. Novick said that among the Streptomycetes, genetic exchange, if sought, is usually found.

Dr. Krimsky said that three categories of organisms can be considered: (1) those organisms known to be pathogens, (2) those organisms known not to be pathogens, and (3) those for which insufficient data exists to determine whether they are pathogens or nonpathogens. He suggested that the language of the proposal should be restricted to permit P2 containment only for known nonpathogens. Dr. Gill said it would be difficult to establish a list of nonpathogens; certain organisms might or might not be included on the list depending on the definition of pathogenicity. She said two Streptomcyes species, currently included in Appendix E, have been reported to cause infections.

Dr. Krimsky proposed that the motion be amended to read as follows:

> "P2 physical containment shall be used for DNA recombinants produced between members of the Actinomycetes group which are known not to be pathogenic to man, animals or plants."

Dr. Brill said he would not accept the amendment. He said hundreds of thousands of Actinomycetes soil isolates had been screened in attempts to isolate antibiotic producers without any known disease having been caused in laboratory workers; past history says that they are safe. Dr. Berns asked why P2 containment was being proposed. Dr. Taylor said Actinomycetes are generally manipulated under P2 conditions; the organisms grow slowly and P2 containment is used to prevent contamination.

Mr. Thornton called the question on the amended language proposed by Dr. Krimsky. By a vote of three in favor, eleven opposed, and three abstentions, the RAC turned down the proposed amendment.

Mr. Thornton then called the vote on the language appearing in the Federal Register. By a vote of sixteen in favor, one opposed, and one abstention, the RAC adopted the language published in the Federal Register, as follows:

> "P2 physical containment shall be used for DNA recombinants produced between members of the Actinomycetes group except for those species which are known to be pathogenic for man, animals or plants."

XVIII. PROTOCOL REQUIRING ASSIGNMENT OF CONTAINMENT LEVELS

Dr. Brill introduced a request (tab 941) from Dr. Dean Taylor of Smith Kline and French Laboratories. Dr. Taylor proposed to transform protoplasts of *Streptosporangium* with a plasmid vector constructed to contain (1) the *E. coli* plasmid pBR322, (2) a *Streptosporangium* plasmid, pS$_g$B-1, (3) an antibiotic resistance determinant from *Streptomyces* species or HV1 approved *Bacillus subtilis* cloning vectors, and (4) flanking sequences from the *Streptomyces* plasmid vector from which the determinant is isolated. A letter from the CDC stating that the species may reasonably be treated as a Class 1 agent had been appended to Dr. Taylor's request.

Dr. Brill moved approval at P2 physical containment. Dr. Scandalios seconded the motion. By a vote of seventeen in favor, none opposed, and one abstention, the RAC adopted the proposal.

XIX. PROPOSED AMENDMENT OF SECTION IV-E-2

Mr. Thornton then called the attention of the RAC to part two of a proposal (tabs 918, 931/11/B) from Dr. Irving Johnson, Eli Lilly and Company, to add a new Section IV-E-2-b to the Guidelines as follows:

> "A permanent subcommittee of the RAC shall be responsible for advising the Director, NIH, on the actions, listed in Section IV-E-1-b-(3)-(d) pertaining to large-scale applications. Submissions that are in compliance with the Guidelines may be recommended by the subcommittee to the Director of NIH for approval. The subcommittee shall also be authorized to consider preliminary plans for large-scale operations and to recommend approval contingent upon completion of the large-scale facility according to those plans. The subcommittee will be responsible for expeditiously processing applications."

Ms. Cason noted that earlier in the meeting (Section XVI) the RAC had voted to establish a large-scale review subcommittee. She moved to table discussion of this proposal. By a vote of fourteen in favor, three opposed, the RAC agreed to table further discussion of the proposal.

XX. MINOR MODIFICATIONS OF LARGE-SCALE PROTOCOLS

Dr. Gottesman said that at the last meeting the Working Group on Large-Scale Procedures had presented a proposal for dealing with minor modifications of previously approved large-scale protocols. She said objections to aspects of the proposal had been raised, and the RAC had voted to defer further discussion until this meeting. She felt any proposal should address the following issues:

(1) What type of expedited procedure should be developed to deal with minor modifications of previously approved large-scale protocols and

(2) What criteria should be applied to determine if a modification is indeed minor.

She said she had not composed specific language to present to the RAC but wished to express her general thoughts, and hoped to obtain a sense of the Committee. She suggested that a formal subcommittee, or a working group, or several reviewers selected by ORDA, could evaluate the applications to determine whether they involve minor modifications of an already approved proposal. If so, the package could be sent to the Director, NIH, for approval without review by the full RAC. She said the individuals responsible for the review would have two responsibilities:

(1) To determine if the modification is indeed a minor modification. If one reviewer does not agree that the modification is minor, the proposal would be evaluated by the full RAC;

(2) If the reviewers unanimously agree the modification is minor, the review group would determine whether the modification significantly affects any containment aspect of the parent proposal. She said the RAC might construct some guidelines in this area, e.g., the general acceptability of deletions, single base changes, additions of short segments that do not lead to production of new products, change to an equivalent vector, etc.

Dr. Gottesman said that while it would be extremely difficult in advance to imagine every possible change and its consequence, it will, in most cases, be obvious to the reviewers when a specific proposed modification is minor. She felt several RAC members involved in evaluating the original parent proposal should be involved in review of any proposed minor modifications. After discussion of various aspects of the proposal, Dr. Gottesman agreed to formulate a specific proposal for consideration at the January 1981 meeting.

XXI. REQUEST FOR CERTIFICATION OF HV1 BACILLUS SUBTILIS HOST-VECTOR SYSTEM

Dr. Brill introduced a request (tabs 921, 931/5) from Dr. David B. Wilson, Cornell University. He said Dr. Wilson requests HV1 certification of a host-vector system based on certified host-components of HV1 Bacillus subtilis host-vector systems and a plasmid, pAB124, isolated from Bacillus stearothermophilus. Dr. Brill said Bacillus stearothermophilus is not a pathogen. He recommended that the plasmid be approved for use with certified HV1 Bacillus subtilis hosts.

By a vote of eighteen in favor, and none opposed, the RAC recommended that plasmid pAB124, isolated from Bacillus stearothermophilus, be certified for use with HV1 certified Bacillus subtilis hosts, as an HV1 host-vector system.

XXII. PROTOCOLS REQUIRING ASSIGNMENT OF CONTAINMENT LEVELS

A. Requests for permission to transform Chlamydomonas reinhardi with E. coli/S. cerevisiae plasmids

Dr. Brill introduced the requests (tabs 910, 911, 931/1) from Dr. John Carbon, University of California, Santa Barbara, and Dr. Stephen Howell, University of California, San Diego. He said these investigators request permission to use the unicellular flagellate Chlamydomonas reinhardi, under P2 physical containment, to clone defined DNA segments derived from E. coli and S. cerevisiae using E. coli/S. cerevisiae hybrid vectors.

Dr. Brill said Chlamydomonas reinhardi is the most studied green alga, and has not been demonstrated to be a pathogen or to produce toxins. He moved approval of the proposal. Dr. Scandalios concurred and added that the organism does not freely exchange DNA with other species.

By a vote of sixteen in favor, none opposed, and one abstention, the RAC adopted the motion to approve the requests.

B. Request for Permission to Transform Candida albicans with E. coli/ S. cerevisiae Hybrid Plasmids

Dr. Maas introduced the requests (tabs 912, 913, 928, 931/2) from Dr. P. T. Magee, Michigan State University and Dr. W. LaJean Chaffin, Texas Tech University. The investigators had requested consideration of the appropriate containment level for the return of Candida albicans DNA to the host of origin. The Candida albicans DNA will be cloned in an EK1 E. coli K-12 or in a laboratory strain S. cerevisiae employing a hybrid E. coli/S. cerevisiae plasmid vector, or the S. cerevisiae 2 micron plasmid.

Dr. Maas said Candida albicans is an opportunistic pathogen and is classified in the proposed revised CDC Biosafety Guidelines as a Class 2 etiological agent. He said the investigators hope to analyze the genetics of the organism using recombinant DNA techniques. Ultimately they hope to elucidate the basis of C. albicans pathogenicity.

Dr. Pinon suggested that P2 containment is adequate. He said Candida does not produce toxins; in addition it is non-invasive. Candida albicans inhabits mucosal membranes, and is considered a normal component of the microflora. Anywhere from 25 to 100% of the population, carry Candida.

Dr. Novick pointed out that Candida can cause disease in individuals receiving broad spectrum antibiotics. Dr. Gottesman said this is essentially a return to host of origin experiment, and felt the relevant question is whether the S. cerevisiae DNA introduced into Candida might contribute to Candida pathogenicity.

Dr. Pinon moved that the experiments proposed by Drs. Magee and Chaffin be permitted at the P2 level of containment. By a vote of fourteen in favor, one opposed, and three abstentions, the RAC approved the motion.

XXIII. INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) SURVEY (Continued)

Dr. Novick requested an opportunity briefly to address the RAC concerning the proposed NIAID evaluation of IBC functions which had been discussed earlier in the meeting (Section IV). He said he would like the RAC to go on record as requesting that the RFP being developed include the following:

(1) An evaluation of MUAs or registration documents submitted to IBCs for compliance with the Guidelines, and

(2) An inquiry of how often physical facilities are inspected by IBCs, and the results of the inspections.

Dr. Goldstein supported Dr. Novick's proposal; he said Dr. Dutton showed the performance of individual IBCs is highly variable. Dr. Gottesman objected to an evaluation of every registration document; she and Dr. Berns thought a sampling procedure might be more appropriate. Dr. Krimsky said that practices for social science research usually involved random sampling. He favored telling the contractor what was to be evaluated, and leaving it to the contractor to use the state of the art of evaluation research to accomplish the task.

It was agreed that Dr. Nutter will be asked to discuss the draft RFP with the RAC at its January 1981 meeting, prior to the RFP being issued.

XXIV. DISCUSSION OF INCIDENT AT UNIVERSITY OF CALIFORNIA, SAN DIEGO

Dr. Goldstein wished to discuss the recent events at the University of California, San Diego (UCSD) concerning an apparent infraction of the

NIH Guidelines. He said he was disturbed that the first report of the problem came up in January and the IBC did not consider the issue until July. Dr. Talbot recounted the history of the incident as detailed in tab 929. He said in January 1980, several graduate students informed Dr. Ian Kennedy at the University of California, San Diego, of their suspicions that the Guidelines were being violated. Only in May did the students speak of this to the biology department chairman. The department chairman obtained a sample of the virus. The sample was sent to the California State Department of Health Services for testing. On July 22, 1980, the results were reported to UCSD; the vial contained Semliki Forest Virus rather than Sindbis Virus. The IBC met on July 30, 1980 and immediately prohibited Dr. Kennedy from continuing recombinant DNA work. Dr. Talbot said that as detailed in tab 929, an NIH committee would meet on October 8, 1980 to review the incident and recommend what response NIH should take. Dr. Talbot said that copies of the final recommendations of that committee will be sent to the RAC.

Dr. Krimsky asked why the apparent Guideline violation had occurred. Dr. Talbot replied that the UCSD IBC report (tab 929) gives their conclusions, and that a reply to this report from Dr. Kennedy had just been received. Dr. Pinon added that Dr. Kennedy had recently resigned from UCSD and that a UCSD departmental committee had also submitted a report to the UCSD Chancellor.

Dr. Krimsky asked about the role of the RAC in dealing with instances of non-compliance with the Guidelines. Dr. Talbot replied that dealing with an individual case is a responsibility of NIH and not the RAC. If from the specific case there arise generic issues suggesting a revision of the Guidelines, the consideration of such revisions would be a RAC function.

XXV. PROTOCOLS REQUIRING ASSIGNMENT OF CONTAINMENT LEVELS (Continued)

C. Proposal to Introduce Genes Cloned in E. coli K-12 into Arabidopsis Plants through the Use of Agrobacterium tumefaciens Carrying an E. coli/Ti Hybrid Plasmid Vector.

Dr. Brill introduced a proposal (tabs 914, 931/3) from Dr. Donald J. Merlo, University of Missouri-Columbia. Dr. Merlo, employing the following protocol, requested permission to introduce E. coli and Arabidopsis genes into Arabidopsis thaliana:

(1) A hybrid plasmid vector, constructed from the E. coli plasmid pBR325 and the origin of replication and transfer genes of Agrobacterium tumefaciens plasmid Ti, will be cloned into E. coli K-12.

(2) *Arabidopsis* DNA will be introduced into the *E. coli*/Ti hybrid plasmid and cloned in *E. coli* K-12.

(3) The thiamine gene of *E. coli* K-12 will be introduced into the *E. coli*/Ti vector carrying *Arabidopsis* DNA, and cloned in *E. coli* K-12.

(4) The hybrid plasmid into which *Arabidopsis* DNA and the thiamine gene have been ligated will be transformed into *Agrobacterium tumefaciens*.

(5) *Agrobacterium tumefaciens* will be used to introduce the *E. coli*/Ti plasmid vector carrying the *E. coli* thiamine gene and *Arabidopsis* DNA into *Arabidopsis* plants.

Steps (1) (2) and (3) are covered by Section III-O of the Guidelines and may be performed under P1 containment. Steps (4) and (5) are covered under entry four of Appendix E and may be conducted under P3 containment. Dr. Merlo requested permission to perform steps (4) and (5) at P1 or P2 containment.

Dr. Brill noted that *Arabidopsis* plants are easily contained. He said *Agrobacterium tumefaciens* is not a serious plant pathogen, as it only invades injured tissues. He moved that the investigator be permitted to perform steps (4) and (5) under P1 containment conditions. Dr. Scandalios concurred.

Dr. Goldstein moved to amend Dr. Brill's motion to require P2 physical containment for steps (4) and (5). Dr. Brill did not accept the amendment. By a vote of four in favor, nine opposed, and three abstentions, the amendment was not accepted by RAC.

Mr. Thornton then called the vote on Dr. Brill's motion. By a vote of twelve in favor, two opposed and three abstentions, the committee accepted the motion to set containment at P1 for steps (4) and (5) of the proposed protocol.

D. Request for Consideration of Appropriate Containment for Experiments Involving *Zymomonas mobilis*

Dr. Maas introduced the request (tabs 922, 931/13) from Drs. B. S. Montencourt and D. E. Eveleigh, Rutgers University, to introduce into *Zymomonas mobilis*, DNA from non-pathogenic *Pseudomonas* strains that had been cloned in *E. coli* K-12.

Dr. Maas said that Zymomonas mobilis is a nonpathogenic anerobic soil bacterium which exchanges genes with Pseudomonas aeruginosa and E. coli. Dr. Barban added that Zymomonas mobilis is frequently found in fermenting plant juices, principally in tropical climates.

Dr. Maas moved approval of the request under P2 conditions. By a vote of seventeen in favor, none opposed, the RAC adopted the motion.

E. Request to Clone Schizosaccharomyces pombe DNA in Schizosaccharomyces pombe

Dr. Brill introduced the request (tabs 930, 931/15) from Dr. Benjamin Hall, University of Washington, to clone Schizosaccharomyces pombe DNA in Schizosaccharomyces pombe using approved HV1 Saccharomyces cerevisiae/E. coli hybrid plasmids as vectors. Dr. Hall requested P1 as the appropriate level of physical containment. He pointed out that Schizosaccharomyces pombe has been the subject of intense genetic studies in the laboratory and traditionally has been used to ferment beverages for human consumption.

Dr. Brill moved to approve the request under P1 conditions. Dr. Pinon concurred. By a vote of seventeen in favor, none opposed, the RAC accepted the motion.

F. Request for Permission to Clone the Tox A Gene of Staphylococcus aureus

Dr. Maas introduced the request (tab 940) from Drs. A. G. Barbour and L. W. Mayer, National Institutes of Health, to clone the pyrogenic exotoxin type A (Tox A) gene of Staphylococcus aureus. The issue is whether this is a prohibited experiment under the Guidelines.

Dr. Maas said Tox A produces fever, enhances susceptibility to E. coli endotoxin, stimulates immune system T cells, and enhances acquired skin reactivity to other antigens. He said Tox A is less potent that botulinum or tetanus toxin. He said Drs. Barbour and Mayer originally requested permission to clone the gene in an HV2 Bacillus subtilis host-vector system, but when informed that no HV2 B. subtilis system had been certified to date, requested permission to clone the Tox A gene in an EK2 system. Dr. Maas noted that certification of an HV2 Bacillus subtilis had been recommended by the RAC at this meeting. He preferred that the B. subtilis host-vector system be used for cloning, as Tox A activates the E. coli endotoxin. He suggested that containment conditions specified for cloning toxin genes should be based on the pharmacological effect of the toxin. He urged caution in concluding that cloning a toxin gene from a mildly pathogenic organism in a non-pathogenic host-vector system will present no

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difficulties. He said toxin expression and potency depend on many variables. He said, however, that he did not consider Tox A to be a potent toxin.

Dr. Berns said pyrogenic Staphylococcus aureus is a potent pathogen. Dr. Nightingale concurred.

Dr. Gottesman said the issue is whether Tox A is a potent toxin; if it is not, P1 + EK1 containment is indicated by the Guidelines for cloning in E. coli K-12. Dr. Novick said he was concerned that toxins which are not "potent" may be cloned at P1 + EK1 containment.

Dr. Gottesman moved that, as the RAC is currently in the process of reassessing the cloning of toxins under the Guidelines, the proposed experiments be approved in E. coli K-12 systems under P3 + EK2 conditions, or at P3 + HV2 with a Bacillus subtilis system. By a vote of seventeen in favor, none opposed, the RAC adopted Dr. Gottesman's motion. (Executive Secretary's Note: The Director, NIH, in his decision limited cloning of the Tox A gene to HV2 Bacillus subtilis host-vector systems under P3 containment for the present time.)

G. Request for Permission to Construct an E. coli/H. influenzae Hybrid Plasmid for Use in H. influenzae

Dr. Gottesman introduced the proposal (tab 959) from Dr. Hamilton Smith, Johns Hopkins University. Dr. Smith wishes to insert an E. coli gene coding for tetracycline resistance (Tn10) into the Haemophilus influenzae plasmid pRSF0885. The plasmid construct would be used in Haemophilus influenza strain Rd to clone Haemophilus genes.

Dr. Campbell moved to approve the experiments under P1 conditions. By a vote of twelve in favor, none opposed, and seven abstentions, the RAC adopted the motion.

XXVI. FUTURE MEETING DATES

Dr. Gartland called the Committee's attention to tab 951 which lists futur meeting dates. He said the meeting which had been planned for December 1981 had been rescheduled to January 1982.

XXVII. CLOSED SESSION

The RAC went into closed session to consider proposals from commercial concerns for scale-up of recombinant DNA experiments.

XXVIII. ADJOURNMENT

The meeting adjourned at approximately 4:40 p.m., Friday, September 26, 1980.

Respectively submitted,

Elizabeth Milewski
Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland Jr.
William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Feb 12, 1981
Date

Ray Thornton
Ray Thornton, J.D.
Chairman
Recombinant DNA Advisory Committee

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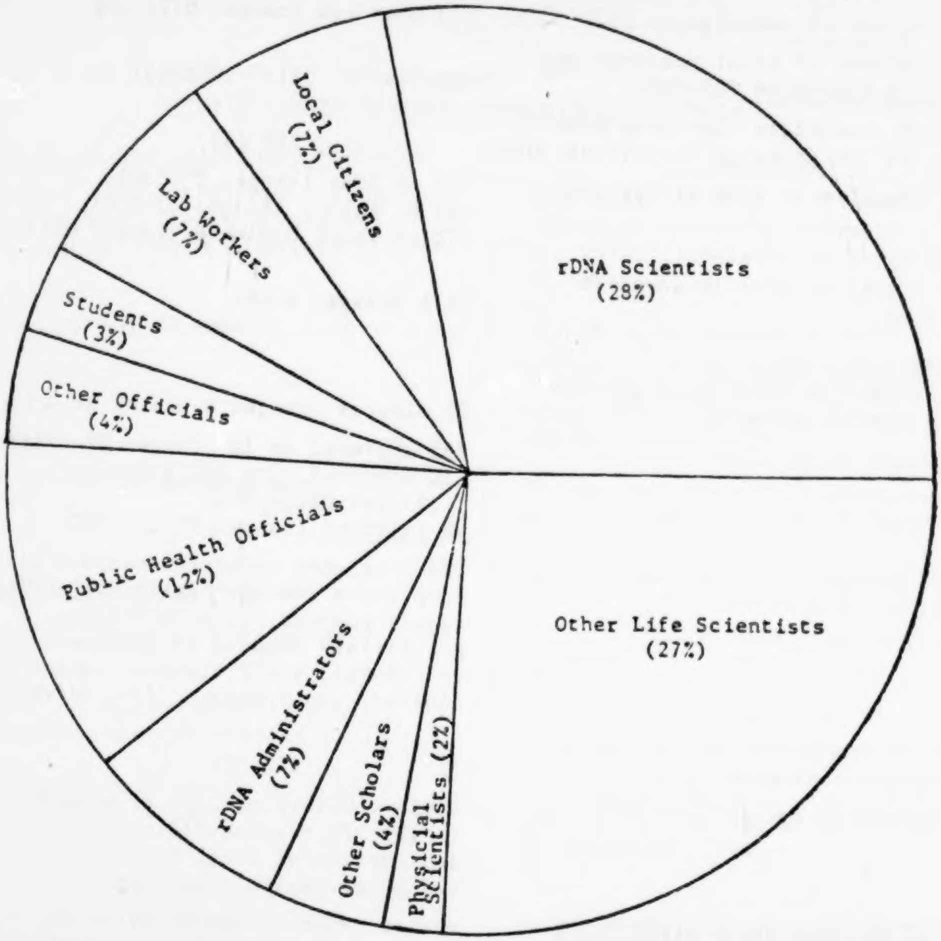
SUMMARY DATA FROM SURVEY OF CALIFORNIA INSTITUTIONAL BIOSAFETY COMMITTEES*

presented by Diana B. Dutton, Ph.D., Stanford University, September 25, 1980

Figure 1. Membership composition of a "typical" IBC in California
(percent of total members per committee in parentheses)



Roster Category	Description	Average number of members: Total	Affiliated	Nonaffiliated
1	rDNA Scientists	3.0	2.6	.4
2	Other Life Scientists	2.9	2.7	.2
3	Scholars in Physical Sciences	.2	.1	.1
4	Other Scholars	.4	.2	.2
5	rDNA Administrators	.7	.7	0
6	Public Health Officials	1.3	.6	.7
7	Other Officials (administrative and government)	.4	.3	.1
8,9	Students (only for academic institutions)	.3	.3	0
10	Lab Workers	.8	.7	.1
11	Local Citizens	.8	0	.8
		10.8	8.2	2.6

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*Responses based on 19 of the 20 IBCs in California, and 45 of the 48 non-affiliated IBC members.

Table A
IBC Structure, Performance, and Assessments

Structure and Functions of IBCs	Responses
IBCs' relation to local governments (Q36)	56% (9) said none, 25% (4) said city officials are IBC members
Average number of meetings in 1979 (Q7)	4.3 meetings (range: 0(2)-15)
Average percent of total meetings not regularly scheduled (Q7a/Q7)	63% (range: 0%(3)-100%(8))
Percent of committees that have some "meetings" by phone or in writing (Q11)	22% (4)
Typical division of time at meetings (Q17)	51% on MUAs (range: 5%-100%) 32% on policy (Range: 0%-100%) 17% on other business (range: 0%(9)-70%)
Average number of MUAs reviewed in 1979 (Q38)	16.4 (range: 1-68)
Scope and Performance	
Average committee time spent per MUA (Q7xQ13xQ17a)/(Q38x100)	32 minutes (range: 6 min.-1 hr. 43 min.)
MUA approval rates (Q39)	73% approved as is (range: 0%-100%(4)) 23% approved with modification (Range: 0%(5)-90%) 4% rejected (range: 0%(11)-40%)
Types of policy and procedural issues considered (Q18)	wide variety: committee composition, compliance checks, health monitoring of female workers, PI eligibility, proprietary aspects of proposals, voting procedures, staff training, grant and contract requirements, fire safety.
Percent of committees with a health surveillance program (Q46a)	28% (5)
Emergency plans for spills (Q49)	varied: safety manuals or materials (n=12), PI responsibility (n=2), designation of local hospital site and MD as health consultant.
Training for laboratory staff (Q50a)	varied: PI responsibility (n=8), environmental health and safety staff conduct training, training plus exam by laboratory manager.
Training for IBC members (Q50b)	varied: none, university course, NIH course, training documents plus discussions plus laboratory tours.
Overall Assessment of IBCs and Guidelines	
Chairs' evaluation of Guidelines regulating IBCs (Q52)	83% said about right 17% said too restrictive
NA members' assessment of whether IBCs are worthwhile (P20)	91% said yes

"Q" and "P" indicate question numbers on chair and non-affiliated questionnaires, respectively.

Table B
Non-affiliated Members: Composition and Role

Who Are the Non-affiliated Members?	Responses
Breakdown of all (48) non-affiliated (NA) members (based on roster categories listed in Figure 1)	31% local citizens, 27% public health officials, 15% rDNA scientists, 10% life scientists, 8% other scholars 8% other
Composition of NA members by committee	1=100% (4/4) rDNA scientists 1=100% (3/3) rDNA and life scientists 1=67% (2/3) rDNA and life scientists 4=50% (1/2) life scientists 13=100% nonscientists
Selection of NA members (Q2)	74% by administrative appointment, 26% other methods (mostly involving appointment)
What Role Do They Play?	
How are committee decisions made (Q14)?	47% by majority vote, 42% by consensus, 10% other
Chairs' evaluation of Guidelines' 20% NA member requirement (Q53)	61% said about right 39% said too restrictive
NA members' self-rating of their role on IBCs (P23)	27% said valuable or very valuable 60% said fairly valuable, 13% said not valuable

Comparing committees with all NA members nonscientists versus 50-100% of NA members scientists:	no NA scientists (12 IBCs)	50-100% NA scientists (7 IBCs)
-average percent committee time spent on MUAs (Q17a)	45%	64%
-MUA approval rates (Q39):		
average percent as is	71%	77%
average percent with minor modification	33%	16%
average percent rejected	5%	2%

Table C
Public Access to IBCs Proceedings and Information

Public Access to IBCs	Responses	
Percent of meetings open to public (Q20)	42% (8) said none, 53% (10) said all, 5% (1) said half	
Percent of committees which announce public meetings (Q22)	0%	
Outside attendance at IBC meetings (Q23)	62% (8) said no one, 23% (3) said 1 person, 15% (2) said 2 people	
Places where minutes are available (Q32c)	23% (3) said library, 85% (11) said with IBC chair or other institutional office	
Chairs' assessments of public meetings (Q31)	56% (9) said desirable 44% (7) said undesirable	
Percent of non-affiliated members who said IBC decisions "always" account adequately for community views (P18), comparing IBCs with no open meetings vs. 50-100% open meetings (Q20)	no open meetings 53%	50-100% open meetings 22%
Proprietary Issues		
Percent of committees which restrict agendas during public meetings (Q28)	29% (2), both for-profit institutions	
Percent with provisions to restrict minutes (Q34)	33% (4), all named potentially patentable results as reason	

U.S. Department of Labor

Assistant Secretary for
Occupational Safety and Health
Washington, D.C. 20210

SEP 24 1980

Dr. Donald Fredrickson
Director
National Institutes of Health
5600 Fishers Lane
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

As you know, both the NIH Recombination DNA Advisory Committee (RAC) and OSHA, among others, have been concerned with how the recombinant DNA industry should be monitored in an attempt to protect workers from any existing or potential hazards which may be peculiar to this type of activity. OSHA has been actively reviewing the ways in which we can fulfill our mandate in this regard.

In recognition of our need to develop a regulatory policy concerning recombinant DNA, representatives of OSHA and NIOSH have been attending the RAC meetings and participating in the meetings of the Interagency Advisory Committee on Recombinant DNA Research and of its Industrial Practices Subcommittee. In addition, NIOSH has undertaken a study of the industry and its potential hazards, as decribed in the attached memorandum, which will provide a basis upon which OSHA regulatory decisions in this area can be developed. We estimate that for completion of the NIOSH study and for implementation of OSHA's subsequent decisions, a period of approximately two years will be required.

I regret that the RAC wishes to terminate its reviews of engineering plans for proposed private-sector DNA operations. Because this would remove one of the existing procedures for promotion of biological safety in these operations, I would like to describe to you how OSHA proposes to deal with recombinant DNA workplaces during the interim period, until OSHA regulatory policy for this industry is developed.

Until formal regulatory decisions are made, OSHA will regulate recombinant DNA operations under its current authority, standards, and guidelines. Thus, OSHA will respond to complaints from workers, unions, or others about recombinant DNA hazards by reviewing the complaints and conducting inspections as warranted. In addition, inspections can also be initiated by OSHA without receipt of a specific complaint if the workplace selection is made in conformity with OSHA's current guidelines on general schedule inspections. If an inspection of a recombinant DNA facility is undertaken, OSHA compliance officers will, consistent with OSHA established practice,

look for violations of existing standards and of the general duty provision (Section 5(a)(1)) of the OSH Act which states, in part, that the employer shall furnish a place of employment free from recognized hazards that are likely to cause serious physical harm. Furthermore, during such an inspection, OSHA may choose to determine whether the employer is in compliance with the RAC recommendations and with applicable sections of the NIH guidelines. OSHA will inform the employer of any observed failures of such compliance and will determine what OSHA action should be taken to bring about worker protection from recombinant DNA hazards at that workplace.

We hope that these procedures by OSHA, coupled with the continued function of the RAC and continued voluntary compliance by industry with RAC recommendations, will provide for safety of the workers in this developing industry.

Sincerely,

Eula Bingham

Eula Bingham
Assistant Secretary
Occupational Safety and Health

federal register

Friday
November 21, 1980

Part VI

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research, Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the January 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724).

EFFECTIVE DATE: November 21, 1981.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496-6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These were reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on September 25–26, 1980. In accordance with Section IV-E-1-b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

This announcement provides background information on the actions including an analysis of correspondence concerning these actions received up to Octoiber 31, 1980.

Immediately following this announcement, there appears in the **Federal Register** a copy of revised NIH Guidelines for Research Involving Recombinant DNA Molecules. These revised Guidelines differ from the previous version of the Guidelines promulgated on January 29, 1980 (45 FR 6724) by incorporating within them: changes in the Guidelines which were recommended at the RAC meeting of March 6–7, 1980, and promulgated on April 14, 1980 (45 FR 25366); changes in the Guidelines which were recommended at the RAC meeting of June 5–6, 1980, and promulgated on July 29, 1980 (45 FR 50524); and changes in the Guidelines which were recommended at the RAC meeting of September 25–26, 1980 and which are discussed in this announcement.

I. Proposal To Introduce Genes Cloned in E. Coli K-12 into Arabidopsis Plants Through the Use of Agrobacterium Tumefaciens Carrying an E. Coli/Ti Hybrid Plasmid Vector

In a letter dated March 21, 1980, Dr. Donald J. Merlo of the University of Missouri-Columbia, requested an evaluation of the containment appropriate for the following protocol:

A. A hybrid vector, constructed from the *E. coli* plasmid pBR325 and the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, will be cloned in *E. coli* K-12.

B. *Arabidopsis* DNA will be introduced into the *E. coli*/Ti hybrid plasmid and clone in *E. coli* K-12.

C. The thiamine gene of *E. coli* will bed introduced into the *E. coli*/Ti vector carrying *Arabidopsis* DNA and cloned in *E. coli* K-12.

D. The hybrid plasmid into which *Arabidopsis* DNA and the thiamine gene have been ligated will be transformed into *Agrobacterium tumefaciens.*

E. *Agrobacterium tumefaciens* will be used to introduce the *E. coli*/Ti plasmid vector carrying the *E. coli* Thiamine gene and *Arabidopsis* DNA into *Arabidopsis* plants.

The RAC discussed Dr. Merlo's proposed protocol at the June 5–6, 1980 meeting. The RAC stated that the first three steps of the proposed experiment are covered by Section III-O of the Guidelines. It was agreed that steps D and E are covered by Appendix E and that P3 containment is indicated. However, as the proposed inserted sequences were well characterized, and as *Arabidopsis* DNA will be returned to *Arabidopsis,* the RAC suggested that containment for steps D and E could be lowered to P2.

Since this had not been published in the **Federal Register** for comment prior to the June 5–6, 1980, RAC meeting, this proposed lowering would, however, have to be published in the **Federal Register** for thirty days of comment and re-considered at the September 25–26, 1980 meeting.

In a letter dated July 22, 1980, Dr. Merlo described the proposed experiments in greater detail and requested that the containment conditions for steps D and E be lowered at least to P2 and preferably to P1.

Dr. Merlo's proposal was published in the August 21, 1980, **Federal Register** (45 FR 55924). No comments were received during the thirty day comment period. The RAC, at its September 25–26, 1980 meeting, once again discussed this issue. A motion to approve the experiments at the P2 level of containment failed by a vote of 4 in favor, 9 opposed, with 3 abstentions. Then, by a vote of 12 in favor, 2 opposed, with 3 abstentions, the RAC recommended approval of the proposed experiments under P1 containment.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

II. Request for Certification of a Bacillus Stearothermophilus Derived Plasmid as the Vector Component of an HV1 Bacillus Subtilis Host-Vector System

Dr. David B. Wilson of Cornell University, in a letter dated May 12, 1980, requested certification of a *Bacillus stearothermophilus* derived plasmid, pAB124, as the vector component of an HV1 *Bacillus subtilis* host-vector system. The HV1-certified *Bacillus subtilis* strain RUB331 would be used as the host component.

The RAC discussed this proposal at the June 5–6, 1980 meeting. It was noted that *Bacillus stearothermophilus* is a non-pathogenic thermophile found in soil near hot springs, on compost, etc. In addition, plasmid pAB124 can be transformed into and maintained in *Bacillus subtilis* by natural physiological methods; pAB124 might, therefore, be considered a plasmid indigenous to *Bacillus subtilis.* The committee agreed that this proposal should be published in the **Federal Register** for comment and acted upon at the September 25–26, 1980 meeting.

Dr. Wilson's proposal was published for thirty days of comment in the August 21, 1980, **Federal Register** (45 FR 55925). No comments were received during this period. The RAC once again discussed the proposal at the September 25–26, 1980 meeting. The RAC recommended approval of the proposal by a vote of 18 in favor, none opposed.

I accept this recommendation, and text has been added to Appendix D of the Guidelines indicating this.

III. Request for Certification of a Bacillus Subtilis Strain as the Host Component of an HV2 Host-Vector System

On March 28, 1980, Dr. William Burke, Jr., of Arizona State University, requested certification of *Bacillus subtilis* strain ASB298 as the host component of an HV2 host-vector system. Dr. Burke's request appeared in the **Federal Register** of April 30, 1980 (4 FR 28907). No comments were received during this period. Additional information was submitted by Dr. Burke in a letter dated May 13, 1980.

Dr. Burke's proposal was evaluated by an *ad hoc* working group and subsequently presented to the RAC at the June 5–6, 1980 meeting. One RAC reviewer said the working group was satisfied with the data presented, and said the single issue not addressed by Dr. Burke concerned the possibility of transfer of genetic information from ASB298 to other *Bacilli* by transformation. After some discussion of this point, the RAC deferred consideration of the proposal pending the submission of additional information on transformation frequencies in soil.

In an addendum dated September 8, 1980, Dr. Burke supplied information on the persistence of ASB298 in sterile soil, and on the frequency of genetic exchange in both soil and under optimized laboratory conditions between strain ASB298 and highly transformable *B. subtilis* strains.

A second notice concerning Dr. Burke's request subsequently appeared in the **Federal Register** of August 21, 1980 (45 FR 55925). No comments on this request were received by the NIH.

The RAC reconsidered this request at its September 25–26, 1980 meeting. On the basis of the strain's characteristics and the frequency of transformation in soil, the RAC recommended, by a vote of 14 in favor, none opposed, and 2 abstentions, that strain ASB298 be certified as the host component of a *B. subtilis* HV2 host-vector system. The RAC specified that ASB898 be used with plasmids certified as the vector components of HV1 *Bacillus subtilis* host-vector systems.

I accept this recommendation, and text has been added to Appendix D of the Guidelines indicating this.

IV. Proposed Amendments Of Section IV–E–2.

In a letter dated April 10, 1980, Dr. Irving Johnson of Eli Lilly and Company, submitted several proposals to amend the Guidelines. One of these proposals would have amended Section IV–E–2 to include the following language:

"Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

This proposal appeared in the **Federal Register** of April 30, 1980 (45 FR 28908). Two comments were received during the thirty day comment period. One commentator stated that:

> "Any attempt to add industrial expertise to the committee * * * is to convert the committee to a self-appointed consensus standards organization."

Another commentator said, "We strongly object to (this) proposal * * *"

The RAC considered this proposal at the June 5–6, 1980 meeting. The committee agreed that, while it would be desirable to have a member with expertise in fermentation technology appointed to RAC, language specifying "representatives of industry" was inappropriate. The RAC agreed that the following amended language should be published for comment and considered at the September 1980 meeting:

> "Members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

In a letter dated July 25, 1980, Dr. Johnson requested that his original language amending Section IV–E–2 be reconsidered at the September 1980 meeting. Dr. Johnson's proposed language and the language constructed by the RAC both appeared for public comment in the **Federal Register** of August 21, 1980 (45 FR 55928). One comment was received during the thirty day comment period:

> "We have long held the view that the RAC membership should include persons versed in scientific aspects of industrial microbiology. As with the rest of the Committee, they should be selected for the excellence of their qualifications. Such persons would complement the present technical expertise of RAC by providing knowledge in areas of fermentation technology and large-scale industrial applications. This expertise differs from that of safety engineers and is concerned with basic biological problems of large-scale technology * * *"

The proposals to amend the language of Section IV–E–2 were discussed by the RAC at the September 25–26, 1980 meeting. At that time, the RAC again felt that appointing fermentation technology expertise to RAC was desirable, but that the proposal language specifying appointment of "representatives of industry" was inappropriate. By a vote of 13 in favor, 2 opposed, and 2 absentions, the RAC recommended approval of the following language:

> "Members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

I accept the intent of the RAC recommendation that RAC "members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production." I do not believe that it is necessary to change the NIH Guidelines or the RAC Charter in order to assure that the intent of the RAC be honored. This can be accomplished through appointment of one or more individuals with appropriate expertise to fill vacancies that periodically occur in RAC membership.

V. Request to Clone Schizosaccharomyces Pombe DNA in Schizosaccharomyces Pombe Using Hybrid Plasmid Vectors

Dr. Benjamin Hall of the University of Washington requested permission to clone *Schizosaccharomyces pombe* DNA in *Schizosaccharomyces pombe* using approved HV1 *Saccharomyces cerevisiae/E. coli* hybrid plasmids as vectors. Dr. Hall pointed out that *Schizosaccharomyces pombe* has been the subject of intense genetic studies in the laboratory and has been used to ferment beverages for human consumption. He requested that P1 be established as the level of physical containment.

This proposal appeared in the August 21, 1980, **Federal Register** (45 FR 55929). No comments were received during the thirty day comment period.

The RAC discussed this proposal at its September 25–26, 1980 meeting, and recommended approval by a vote of 17 in favor, none opposed.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

VI. Proposed Amendment of Appendix E of the Guidelines

In a letter dated July 28, 1980, Dr. Fritz Reusser of The Upjohn Company requested that two sections of Appendix E be amended. These sections currently read as follows:

- "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.
- "*Streptomyces coelicolor* can be used as a host for the cloning of DNA derived from *subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as a vector any plasmid indigenous to *Streptomyces coelicolor* or able to replicate in that host by natural biological mechanisms."

The proposed revision would permit additional *Streptomyces* species to be utilized in these experiments. The species to be added are already included in Appendix A, Sublists C and D, as two groups of *Streptomycetes* known to exchange DNA by physiological means. Dr. Reusser noted that these species are not pathogenic for humans, animals or plants. The proposed revised sections of Appendix E would read as follows:

- "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus*, and *S. venezuelae* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

- "*Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus*, and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis*, under P2 conditions, using as vectors any plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

The proposed appeared in the **Federal Register** of August 21, 1980 (45 FR 55928). No comments were received concerning the proposal during the thirty day comment period. The RAC evaluated the proposal at the September 25–26, 1980 meeting, considering and voting separately on each of the two sections proposed to be amended.

With regard to the first section, by a vote of 14 in favor, none opposed, and 3 abstentions, the RAC recommended that the proposed amended language be adopted, i.e.:

- "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions."

I accept this recommendation, and this section of Appendix E has been so amended.

The RAC then considered the second section proposed to be amended. During the discussion it was suggested that the language be modified to specify the use of "nonconjugative" plasmid vectors. By a vote of 15 in favor, none opposed, and 2 abstentions, the RAC recommended approval of the following modified language:

- "*Streptomyces coelicolor S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis*, under P2 conditions, using as vectors any nonconjugative plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

Subsequent to the RAC meeting, two comments on this amended language were received by the NIH.

One commentator wrote that the proposal:

> "* * *would have the effect of reversing a previous decision by the RAC and increasing the level of containment required for experiments that have been permitted under P2 conditions for some time. Thus, the amendment is a 'major action'; it does not seem appropriate that it be made part of the Guidelines in the absence of a 30-day period providing an opportunity for workers in the field and others to offer comments on it.
>
> "It should also be noted that the concept of 'conjugative' versus 'non-conjugative' plasmids has been derived from enteric bacteria and may not apply to a wide variety of other bacterial species. For example, the mechanism for genetic exchange in *Streptomyces* species is not fully understood; it may be a consequence of hyphal fusion that permits transfer of plasmid and chromosomal genes. If this is true, it may never be possible to isolate a 'non-conjugative' plasmid *per se* * * *"
>
> "Since all of the *Streptomyces* species that exchange genetic information with *S. coelicolor* are free-living non-pathogenic soil organisms—and since the change in the Guidelines originally proposed under Action 916 involved genes from only non-pathogenic, well-characterized donor bacteria, I urge your approval of the original proposal without the amendment."

The other commentator wrote:

> "I would like to see this amended proposal published for public comment and put to a second vote at the next RAC meeting before you approve this action. The amended proposal failed to receive sufficient consideration of the following points. First of all, in no previous actions regarding *Streptomyces* species was any consideration given during the deliberations to establish levels of physical containment as to whether the vectors were capable of mediating exchange of genetic information. Therefore, this action by the RAC is more restrictive than previous actions and, in my opinion, is inconsistent with the intent of the RAC. In fact, experiments can be carried out at P2 levels of containment in which *B. subtilis, E. coli* K–12, or *S. aureus* vector DNA is cloned into *S. coelicolor* hosts using vectors capable of self-mediated exchange in accordance with Appendix E of the current NIH Guidelines.
>
> "Second of all, the use of the term 'non-conjugative' is inappropriate because the actinomycetes do not exchange genetic information by the process of conjugation as it is known for eubacteria. Rather actinomycetes exchange genetic information by heterokaryosis. In some experiments, the frequency of genetic exchange can be altered by the presence of plasmids and some plasmids can mediate their own transfer to new hosts.
>
> "I feel that to restrict cloning in the actinomycetes to using 'non-conjugative' or non-self-transmissible vectors would be unduly harsh and would severely restrict research with this valuable group of organisms. Considering the level of potential hazard (which I consider minimal) and the potential benefit (which I view as considerable), P2 containment is more than sufficient protection regardless of the vectors employed."

Taking into consideration these comments, I am deleting the requirement for use of nonconjugative plasmids in these systems. If anyone wishes to propose a requirement for use of nonconjugative plasmids in these systems, it will be published for comment in the **Federal Register** as a proposed major action for consideration at a future RAC meeting.

Accordingly, the relevant section of Appendix E has been amended to read as follows:

- "*Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus*, and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis*, under P2 conditions, using as vectors any plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

VII. Request for Consideration of a Proposal to Clone the Tox A Gene of Staphylococcus Aureus

In a memorandum dated July 29, 1980, Drs. Alan G. Barbour and Leonard W. Mayer of the Laboratory of Molecular Structure and Functions, Rocky Mountain Laboratory, National Institute of Allergy and Infectious Dieases, requested an assessment of the containment levels appropriate for cloning the *Staphylococcus aureus* pyrogenic exotoxin type A (Tox A). Drs. Barbour and Mayer indicated that they would prefer to clone the Tox A gene in an HV2 *Bacillus subtilis* host-vector system (since one of the activities of Tox A is enhancement of the toxicity of *E. coli* endotoxin) but, if no HV2 *B. subtilis* host-vector system were available, they requested permission to clone the Tox A gene in an EK2 *E. coli* K–12 host-vector system.

The RAC took this request under consideration at the September 25–26, 1980 meeting. The major determination for the RAC to make was whether Tox A is a "potent toxin" under Section I-D-2 of the Guidelines. It was noted that the lethality of Tox A for animals is not great. The RAC by a vote of 17 in favor, none opposed, recommended that the cloning of the Tox A gene be permitted under P3 containment with either an HV2 *B. subtilis* or an EK2 *E. coli* K–12 host-vector system.

The scientists who submitted this request, noting that one of the activities of Tox A is enhancement of the toxicity of *E. coli* endotoxin, said they preferred to work in *B. subtilis* rather than *E. coli* if an HV2 *B. subtilis* host-vector system were available. Since an HV2 *B. subtilis* system is in fact now certified (see item III of this announcement) I am approving the cloning of the *Staphylococcus aureus* Tox A gene in an HV2 *Bacillus subtilis* host-vector system at P3 containment. Text has been added to Appendix E indicating this.

VIII. Request for Permission to Transform Chlamydomonas Reinhardi With E. Coli/Saccraromyces Cerevisiae Plasmids.

The RAC at its September 25–26, 1980 meeting considered similar requests from Dr. John Carbon of the University of California, Santa Barbara, and Dr. Stephen Howell of the University of California, San Diego, to introduce *E. coli-Saccharomyces cerevisiae* hybrid plasmids containing defined DNA segments into *Chlamydomonas reinhardi* under P2 conditions. The host is a eukaryotic, unicellular photosynthetic green algae. *C. reinhardi* is non-pathogenic, produces no known toxin, and is not known to exchange genetic information with other organisms.

This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the thirty day period for comment.

A motion to approve these experiments at the P2 level of containment was passed by a vote of 16 in favor, none opposed, and one absention.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

IX. Request For Permission To Transform Candida Albicans With E. Coli-S. Cerevisiae Plasmid.

The following notice appeared in the **Federal Register** of August 21, 1980:

> *Request for permission to transform Candida albicans with E. coli-S. cerevisiae plasimids.* Dr. P. T. Magee of Michigan State University, and Dr. W. LaJean Chaffin of Texas Tech University, have requested consideration of the appropriate containment level for the return of *Candida albicans* DNA to the host of origin. The *Candida albicans* DNA will be cloned in *E. coli* K–12 or in *Saccharomyces cerevisiae* employing a hybrid plasmid vector derived from *E. coli* K-12-S. *cerevisiae* or the yeast 2 micron plasmid.

During the thirty day comment period, no responses were received.

The RAC discussed this proposal at its September 25–26, 1980 meeting. It was noted that *Candida albicans* is a normal inhabitant of the flora of man. It can be a pathogen in compromised individuals but does not produce a toxin. This is basically a return of DNA to host of origin type of experiment, with the intermediate host being either *E. coli* K–12 or *Saccharomyces cerevisiae*.

The RAC by a vote of 14 in favor, 1 opposed, and 3 absentions recommended approval of this proposal at the P2 level of containment.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

X. Proposal To Transform Haemophilus Influenzae With E. Coli/H. Influenzae Hybrid Plasmid

The RAC at its September 25–26, 1980 meeting considered a proposal from Dr. Hamilton Smith of the John Hopkins University to insert an *E. coli* Tn10 tet^R gene into a naturally-occurring *Haemophilus* plasmid and to use the hybrid plasmid to transform *H. influenzae* Rd, a nonpathogenic strain. It was noted that a tetracycline resistance gene found naturally in *Haemophilus* is genetically related to the Tn10 tet^R gene of *E. coli*. A motion to approve these experiments at the P1 level of containment was passed by a vote of 12 in favor, none opposed, with 7 abstentions.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XI. Proposed Exemption For Streptococcus Sanguis And Streptococcus Pneumoniae

A request submitted by Dr. Walter Guild of Duke University Medical Center that *Streptococcus sanguis* and *Streptococcus pneumoniae* be considered as natural exchangers of DNA under the exemption category of Section I–E–4 and Appendix A of the Guidelines was considered by the RAC. This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the 30 day period for comment prior to the September 25–26, 1980 meeting.

The evidence presented by the investigator demonstrated that these organisms exchange genetic material in both directions by the natural process of transformation. The RAC recommended by a vote of 16 in favor, none opposed, and 1 absention that a new sublist be added to Appendix A of the Guidelines exempting recombinant DNA experiments between *Streptococcus sanguis* and *Streptococcus pneumoniae*.

I accept this recommendation and these two organisms have been added as sublist F of Appendix A.

XII. Request For Permission To Incorporate Recombinant DNA In Zymomonas Mobilis

The RAC considered a request from Drs. B. Montenecourt and D. Eveleigh of Rutgers University to permit the cloning of DNA derived from *Pseudomonas* strains that are non-pathogenic to animals or plants in an *E. coli* K–12 host, followed by transfer of the recombinant DNA into *Zymomonas mobilis*.

This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the 30 day comment period.

The RAC by a vote of 17 in favor, none opposed and with no abstentions recommended approval at the P2 level of containment for the proposed experiments.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XIII. Request To Transform Protoplasts of Streptosporangium With Recombinant DNA

The RAC at its September 26, 1980 meeting considered a proposal to transform protoplasts of *Streptosporangium* with a hybrid plasmid containing pBR322 plus a *Streptosporangium* plasmid into which have been incorporated specified DNA segments from *Streptomyces* species or an HV1 approved *Bacillus subtilis* cloning vector.

It was noted that members of the *Streptosporangium* genera are soil bacteria and have never been implicated in any human, animal, or plant diseases. Further, these nutritionally fastidious organisms have not been reported to produce toxins harmful to humans.

The motion to approve these experiments at the P2 level of containment was passed by a vote of 17 in favor, none opposed, and 1 abstention.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XIV. Proposed Revision of Subsections of Section III–C–1–e

A notice appeared in the **Federal Register** of January 31, 1980 concerning proposed revision of Section III–C–1–e, and its subsections. It was recommended that Section III–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e–(1)–(b), of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The RAC, at its March 6–7, 1980 meeting, recommended adoption of Section III–C–

1–e, III–C–1–e–(1), and III–C–1–e–(1)–(a) as published in the **Federal Register** of January 31, 1980, with certain modifications in Section III–C–1–e–(1)–(a).

The Director, NIH, accepted this recommendation and promulgated the following sections in the **Federal Register** of April 14, 1980:

> "III–C–1–e. All Viral Vectors.
>
> "III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:
>
> "III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses form a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of the genome."

At its March 6–7, 1980 meeting, the RAC voted to defer consideration until the June 5–6, 1980 meeting of the new Sections III–C–1–e–(1)–(b) and III–C–1–e–(1)–(c) as proposed in the **Federal Register** of January 31, 1980, and requested that a working group develop additional information. Accordingly, a working group met on May 13, 1980, during the annual meeting of the American Society for Microbiology in Miami Beach, Florida. The report of the working group was considered briefly at the June 5–6, 1980 RAC meeting when it was decided that it would be considered again by the RAC at its September 25–26, 1980 meeting.

The Working Group discussed the question of the appropriate containment conditions for experiments involving recombinant DNA molecules containing less than two-thirds of the genome of any eukaryotic virus which may be rescued with helper virus. On the basis of the consensus of the virologists, the following recommendation was proposed, as a revision of Section III–C–1–e–(1)–(b) of the Guidelines:

> "III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents (1)."

This proposal was published for comment in the **Federal Register** of August 21, 1980. During the thirty day comment period no responses were received.

At the September 25–26, 1980 meeting, the RAC discussed this proposal again. Some members of the committee expressed concern whether the rescue of a defective recombinant virus with helper virus could result in the formation of a virus with an altered host specificity or increased pathogenicity. It was noted that the working group of virologists previously discussed this point, and reached the conclusion that the recombinant virus would pose no greater biohazard than the wild type virus used as a helper. The consensus of the working group was that the level of containment required for rescue of the recombinant virus should correspond to the same level of biohazard as the helper virus as determined by the CDC classification.

The motion to accept the proposed revision of subsection III–C–1–e–(1)–(b) was passed by a vote of 10 in favor, 5 opposed, and 1 abstention.

I accept this recommendation, and Section III–C–1–e–(1)–(b) has been so modified. This modification justifies two further changes in the Guidelines. First, new text has been added to Section III–C–1 to advise the reader of the relationship of the subsections of Sections III–C–1–a, III–C–1–b, III–C–1–c, and III–C–1–d of the Guidelines to the subsections of Section III–C–1–e. Also, Table IV of the Guidelines has been eliminated.

XV. Proposed Containment For Cloning Between Members of the Actinomycetes Group

Dr. Dean Taylor of the Smith Kline and French Laboratories, proposed that the third entry in Appendix E of the Guidelines be modified to read:

> P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for those species which are known to be pathogenic for man, animals, or plants.

This proposal was made previously by the RAC Working Group on Prokaryotic Host-Vectors Other Than *E. coli* and appeared in the **Federal Register**, April 13, 1979 (44 FR 22316). The RAC considered the proposal at its May 21–23, 1979 meeting and recommended to restrict this so that it did not include the entire *Actinomycetes* group but rather only the genera *Streptomyces* and *Micromonospora*. The Director, NIH, accepted this recommendation and the action was published in the **Federal Register**, July 20, 1979 (44 FR 42916), and appears as the third entry in Appendix E of the Guidelines.

This proposal was published for comment in the **Federal Register** of August 21, 1980. During the 30 day comment period, no comments were received.

During the RAC discussion of this proposal it was noted that the family of *Actinomycetes* include many genera that are not pathogenic for man, animals, or plants. The microorganisms in this group are mainly found in soil, and are of medical and industrial importance. They produce ninety percent of the antibiotics used in medicine. Although some members of the group are parasitic, most do not cause disease, or are marginal pathogens.

A motion to restrict the proposal to members of the *Actinomycetes* group which are known not to be pathogenic for man, animals, or plants, failed by a vote of 3 in favor, 11 opposed, with 3 abstentions.

A motion to accept the proposal as published in the **Federal Register** on August 21, 1980, passed by a vote of 16 in favor, 1 opposed, with 1 abstention.

I accept this recommendation, and the third entry in Appendix E of the Guidelines has been so amended.

XVI. Changes in Registration Requirements

Dr. Maxine Singer, National Cancer institute, National Institutes of Health, proposed a series of changes in the administrative requirements specified by the Guidelines. The proposal would elimnate NIH review, registration, and approval for all experiments assigned containment conditions in the Guidelines.

In submitting the proposal, Dr. Singer stated:

> When the Guidelines were first promulgated, many arguments appeared to dictate a complex system of review and approval prior to the initiation of experiments. Regardless of how one views the wisdom of the earlier decisions, the multi-level system with its emphasis on central review at the NIH is now clearly unnecessary, inhibitory, expensive, and conterproductive in relation to the respect accorded the containment recommendations. By now, Institutional Biosafety Committees have accumulated sufficient expeience with and knowledge of the Guidelines to operate as independent review groups. Safety Officers now function in many research institutions. Furthermore, the good laboratory practices mandated by the Guidelines have become routine practice in many laboratories. Indeed, confidence in the efficiacy of Guidelines has been based primarily on compliance by individual investiators within their own laboratories. The administrative changes that accompanied the revised containment levels for experiments with *E. coli* K–12 and *S. cerevisiae* earlier this year recognized that central review and approval were no longer necessaryily desirable or useful. The present proposal is an extension of that idea.

In brief, the proposal eliminates central review, registration, and approval for all experiments assigned containment conditions in the Guidelines. Investigators would be required to notify their Institutional Biosafety Committees prior to the initiation of experiments and those committees would be responsible for reviewing the registration documents for consistency with the provisions of the Guidelines and advising the investigators of necessary changes. No Memoranda of Understanding would be required. IBCs and investigators would still be expected to petition the ORDA regarding experiments not explicitly covered by the Guidelines, experiments requiring case-by-case review and experiments involving exceptions to the provisions (or prohibitions). The NIH and the RAC would function as policy making elements with responsibility to change the Guidelines as required, to evaluate containment requirements for experiments not explicitly covered by the Guidelines, and for consideration of exceptions.

Seven years have passed since the scientific community first raised questions regarding recombinant DNA experiments. The ignorance of those early years has been supplanted with a wealth of experience and information. Just as the early stringent Guidelines were promptly adopted in response to the ignorance, we must now respond just as promptly to the current more realistic appraisals.

The proposed revisions were published in the **Federal Register** of August 21, 1980, for comment, as Item 8 of the notice. Specific changes were proposed in many different places in the Guidelines, specified under subheadings "A" through "X" within Item 8.

Seventeen letters were received commenting on the proposal. Sixteen commentators supported its adoption. One commentator stated:

> Maxine Singer's initiative (**Federal Register**, August 21, 1980) to have the NIH Guidelines fall primarily under local control is both wise and timely. Decisions about scientific research are best left to the investigtors both for efficiency and as a matter of fundamental principle. Only in exigency, should this ideal be violated. Perhaps we've passed through such an occasion with R-DNA research. However, it is now the time to return to the more normal situation. Since work with recombinant DNA has proven to be very safe and very important, dual review at the national and local levels serves no good purpose.
>
> As one involved from the beginning in the R-DNA issue, I've always felt that the 'Guidelines' should be guidelines; they turned into regulations. With the proposed change, they will revert and also the unfortunate quasi-regulatory role that the NIH has had to play will begin to disappear.

Another commentator said:

> I think the IBC mechanism has proven its effectiveness in interpreting and enforcing the letter and intent of the Guidelines governing recombinant DNA research. I do not see how the practices of submission, obtaining and recording, etc., of MUA's particularly the requirements for annual submissions of MUA's for each continuing NIH grant or for applications for new grants, contributes to the safety of research. An updated statement of work in progress from each investigator to the IBC's would be significantly simpler to administer and would ensure as much real safety as the present system.

Another observed:

> I agree with her that the function of review, registration, and monitoring of research workers falling into categories explicitly mentioned in the Guidelines can be effectively administered by the local Institutional Biosafety Committees. This would reduce the workload for ORDA allowing it to spend more time determining policy and revising the Guidelines according to requests or to new data. It would simultaneously ease the bureaucratic workload for the researcher without jeopardizing the effectiveness of the Guidelines. Not only are the procedures described in the Guidelines now standard practice among most laboratories, as pointed out by Dr. Singer, but I feel that the local Institutional Biosafety Committees are in much better position to evaluate compliance with the Guidelines. Because of their past experience with the Guidelines, I feel that these committees are ideally suited to take over this important administrative role.

One commentator made the following observation about Institutional Biosafety Committees:

> Institutional Biosafety Committees clearly seem able to operate as independent review groups, and the laboratory practices set forth in the Guidelines have now become part of the standard operating procedures of laboratories working in the field. In other fields of biological research, safety procedures are implemented entirely at the local level, even when known biohazards are involved. It is no longer necessary to have a unique multi-level system in an area where seven years of extensive experience has shown the absence of a unique hazard.

Another commentator said the following about central review:

> Elimination of central review at NIH for experiments classified in the Guidelines will save a great deal of time for both the investigator and the reviewers at NIH. I believe the experience of the past six years justifies a change toward an administrative arrangement long successfully practiced in the handling of proven microbial pathogens, namely national guidelines enforced by local biosafety committees.

One commentator made the following observation about the current system of NIH review:

> The present complex system of review on both local and Federal levels is no longer necessary. It is wasteful of time, effort, and money. It is, in fact, counterproductive because bureaucratic requirements seen by investigators to be clearly unnecessary lead to disrespect for regulations that should be respected. Institutional Biosafety Committees should, in my opinion, be fully capable of monitoring laboratory practices and containment levels specified by the Guidelines.

Another letter included the following remark:

> I strongly endorse the proposed changes because I believe that the risks of this research are now clearly minimal and because the bureaucracy and consequent wasted time are truly detrimental to progress in an area that promises to be of great benefit to mankind.

The Chairman of one IBC reporting the unanimous endorsement of his committee stated:

> We feel strongly that safety is solely a function of the effectiveness of the local institutional practices, on the part of the PI's and the IBC, in complying with and enforcing the Guidelines. The submission of MUAs adds nothing but expensive and time-consuming paperwork.

The one letter against the proposal stated the following:

> At present, we do not believe that there are good reasons for changing the registration requirements. Although present procedures demand some extra paperwork, they permit centralized keeping of files which could be of significant use in reviewing the extent of work on recombinant DNA and its possible hazards. Somebody should know what is going on.

The RAC discussed the proposal at length at its September 25–26, 1980 meeting. Dr. Singer, who attended this portion of the meeting, summarized the proposal and presented her arguments for adopting it. It was noted that the proposal has two major aspects, which could be acted upon separately. The first aspect is that it would eliminate NIH review of protocols for which containment levels are specified by the Guidelines. The second aspect is that the proposal would eliminate the requirement for pre-review by the local IBC prior to initiation of all experiments for which containment levels are specified by the Guidelines (Prior review is already not required for most experiments). Some members of the RAC felt that adoption of the proposal should be delayed until a broad-based survey of the functioning of IBCs is completed. Some members of the RAC felt that pre-review of all experiments by the IBC should not be eliminated at the present time. The RAC then voted separately on a four-part motion proposed by Dr. Gottesman.

The RAC voted 15 in favor, 3 opposed, with no abstentions, to eliminate the requirement for NIH review of IBC decisions on any experiments for which containment levels are specified in the Guidelines.

The RAC voted 12 in favor, 5 opposed, with 1 abstention, to defer consideration of eliminating pre-review of experiments by the IBCs until the frequency of principal investigator error in selecting the appropriate containment levels has been determined.

The RAC voted 17 in favor, none opposed, with 1 abstention, that IBCs keep records of recombinant DNA research done in their institution, including a record of the frequency of errors in classificaion of experiments by the principal investigator.

The RAC voted 15 in favor, 3 opposed, with no abstentions, that IBCs no longer need register with NIH recombinant DNA experiments for which containment levels are specified in the Guidelines.

I accept these recommendations.

The four-part motion passed by the RAC indicated the intent of the RAC to recomment acceptance of certain concepts in the proposal by Maxine Singer (i.e., elimination of the requirement for IBC registration with, and NIH review of, experiments for which the containment levels are specified in the Guidelines), but to defer recommendation of other concepts (i.e., the proposal to eliminate pre-review by the IBC for these experiments).

As noted earlier, the proposal by Maxine Singer published as Item 8 in the **Federal Register** of August 21, 1980, consisted of proposed changes in many different places of the Guidelines, specified under subheadings "A" to "X". Following the RAC meeting, I asked a four-person group to revise these proposed changes in order to translate the four-part general motion of the RAC into specific Guideline changes. The group (consisting of Dr. Bernard Talbot, my Special Assistant, Dr. William Gartland, Director of the Office of Recombinant DNA Activities, Dr. Maxine Singer, originator of the proposal, and Dr. Susan Gottesman, RAC member and originator of the four-part motion passed by the RAC) unanimously agreed on wording which translates the intent of the RAC into specific changes in the Guidelines. I have accepted their recommendations.

The result is that some of the changes "A" through "X" originally proposed by Dr. Singer have been accepted as proposed; some have been rejected, thereby leaving the relevant portions of the Guidelines unchanged; and some have been further modified so that particular sections now are different from both the Guidelines prior to the Singer proposal and from the Singer proposal.

Specifically, the sections where the changes proposed by Dr. Singer have been accepted are: "E"—Deletion of Section IV–C–3; "F"—Deletion of Sections IV–D–1–C and IV–D–1–C–(1) through IV–D–1–C(5); "G"—Deletion of Section IV–D–1–d; "H"—Amendment of Section IV–D–2–f; "I"—Amendment of Section IV–D–2–h; "K"—Amendment of Section IV–D–3–b; "L"—Amendment of Section IV–D–3–f; "P"—Amendment of Section IV–D–5–c–(3); "U"—Deletion of Section IV–E–3–b, IV–E–3–c–(1), IV–E–3–c–(2), and IV–E–3–c–(3); "V"—Deletion of Section IV–E–4–a; and "W"—Deletion of Sections IV–F–1 and IV–F–2.

The proposed changes which have been rejected thus leaving the Guidelines as they were previous to the Singer proposal are: "Q"—Heading of Section IV–D–5–d; "R"—Heading of Section IV–D–5–e; and "T"—Proposed deletion of Section IV–E–1–b–(3)–(e).

The proposed changes which have been forther modified so that the sections now read differently from the Guidelines prior to the Singer proposal and from the Singer proposal are: "A"—Section III; "B"—Section III–0; "C"—Last sentence of Section III–A–3–a; "D"—Last sentence of Section III–A–3–b; "J"—Section IV–D–3–a; "M"—Section IV–D–5–a–(1); "N"—Section IV–D–5–b–(4); and "O"—Section IV–E–5–b–(5).

In addition, Sections IV–F–3, IV–F–4, and VI–C, dealing with registration, have been eliminated, as has the proposed Section IV–D–5–e–(6).

One consequence of these changes is that MUAs need no longer be filed with NIH and the term "MUA" has been deleted from the Guidelines.

One part of the RAC four-part motion called for IBCs to keep records of recombinant DNA research done in their institution, including a record of the frequency of errors in classification of experiments by the principal investigator. This requirement for record keeping will be specified in the revision of the Administrative Practice Supplement to the NIH Guidelines to be issued in November 1980.

During the RAC discussion, the concept of requiring the IBCs to report annually to NIH on all recombinant DNA research being done at the institution was rejected. NIH is sponsoring a meeting of IBC Chairmen on November 24–25, 1980. The issue of possibly reporting annually to NIH will be discussed at that meeting. Following that discussion, I will again review this issue.

It should be emphasized that NIH remains responsible for specifying containment conditions for all experiments not explicitly covered by the Guidelines. This includes experiments requiring case-by-case review and exceptions to the provisions or prohibitions of the Guidelines. Principal investigators must petition NIH for consideration of such proposed experiments. NIH will follow existing procedures in such cases and will notify the principal investigators as before. No such experiments are to be approved by the IBCs until containment conditions have been set by the NIH. No such experiments are to be initiated by PIs until appropriate registration documents have been submitted to and approved by the IBC. The registration documents should include the NIH statement of containment conditions.

XVII. Procedures For Review of Large-Scale Experiments

Section I–D–6 of the Guidelines prohibits "large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNA's, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established."

Section IV–E–1–b–(3)–(d) of the Guidelines states that the Director, NIH, is responsible for "authorizing under procedures specified by the RAC, large-scale experiments (i.e., more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Part VI of the Guidelines, "Voluntary Compliance," encourages institutions not otherwise covered by the Guidelines to follow the standards and procedures set forth in the Guidelines.

At its September 1979 meeting, the RAC adopted the following procedures to be followed by applicants proposing to exceed the 10-liter limit:

"Application Procedures for Large-Scale Recombinant DNA Experiments

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10-liter limit.

"c. A specification of the total volume of the fermenter to be used.

"d. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"e. A description of the applicant's laboratory practices, containment equipment, and facilities relevant to the containment of large volumes of culture.

"f. Evidence of the applicant's or applicant institution's previous experience in handling large volumes of culture. Applicants should exhibit knowledge of state-of-the-art procedures for working with large volumes of microorganisms.

"g. A description of procedures to be employed for the inactivation and disposal of large volumes of culture.

"h. A description of procedures for containing and inactivating accidental spills, should they occur.

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

At recent RAC meetings, there have been extensive discussions of the role of the RAC and NIH in the review of large-scale proposals submitted by industry. (Minutes of RAC meetings are available from ORDA.) At the June 1980 meeting, the RAC passed a motion by a vote of seventeen to zero with one abstention that the following proposal be published in the **Federal Register** for consideration at the September 1980 meeting:

"The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. The RAC will determine if a given recombinant DNA-containing strain is rigorously characterized and the absence of harmful sequences established. Such a determination shall include specification of the containment level (P-LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industry scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC."

This proposal was published for comment in the **Federal Register** of August 21, 1980. There it was pointed out that if the proposal were accepted, it would have the effect of changing the application procedures to read as follows:

"Application Procedures for Large-Scale Recombinant DNA Experiments

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10-liter limit.

"c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"d. Specification of the P-LS level proposed to be used as defined in the NIH *Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules.* (**Federal Register**, April 11, 1980).

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

During the comment period, two comments were received.

One commentator stated:

> I regret that the RAC wishes to terminate its reviews of engineering plans for proposed private-sector DNA operations.

Another letter stated:

> We agree with the RAC proposal passed in June 1980, to exclude regulatory functions dealing with industrial scale-up. We believe that the current mission of the RAC is appropriate and that its present constitution, representing scientific and public interests, is not well suited for a regulatory role. Other bodies of the federal government already have the mandate to carry out regulatory functions and should acquire necessary resources and expertise.

The proposal was discussed at length at the September 25-26, 1980 RAC meeting.

Dr. Krimsky said that although at previous RAC meetings he had argued strongly for proposals like this, he had now changed his mind, because he had come to realize that such pre-review as the RAC has been performing is unique, and that no other agency would conduct such a review. He proposed, as an alternative proposal, that there be established a subcommittee of the RAC made up of some members of the RAC and some members of NIH staff with expertise in facilities and technologies, and that the subcommittee request representation from the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), the Center for Disease Control (CDC), and the Environmental Protection Agency (EPA). In this proposal, the subcommittee would review engineering and technology, and its review would be transmitted directly to the Director, NIH. After discussion, this alternative motion was withdrawn from consideration for the time being, with the understanding that it would be reconsidered later in the meeting.

During further discussion of the proposal as published in the **Federal Register** of August 21, 1980, Dr. Fedoroff indicated her support for eliminating RAC review of the details of physical containment for individual large-scle proposals, as is already the case for small-scale research. Dr. King indicated her support of the proposal as an acceptable compromise.

Following further discussion, the RAC voted 12 in favor, 5 opposed, with one abstention, to adopt the proposal as published in the **Federal Register** of August 21, 1980. I accept this recommendation and procedures for its implementation are published at the end of this section.

Later in the meeting, Dr. Krimsky introduced the following motion to establish a large-scale review subcommittee:

"An industrial review subcommittee of the RAC shall be established with the responsibility for advising the Director on procedures and facilities design pertaining to applications for large-scale operations.

"After the full RAC has reviewed the biological containment requirements for a large-scale proposal, the subcommittee shall examine the applicant's plans for large-scale operations and issue recommendations to the Director on plant design, health surveillance and environmental monitoring. The Director shall advise institutions of recommended design parameters and operational procedures. The determination shall not be construed as NIH certification of industrial operations.

"The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA."

Dr. Fedoroff expressed concern about delays if first the full RAC and then subsequently a subcommittee were to be involved in the review process. Others agreed. Dr. Gottesman said that she was opposed to continuation of review of equipment design in individual applications by either the full RAC or subcommittee. Dr. Logan of OSHA, speaking in behalf of the proposal, said that the delays need not be serious and that such subcommittee review would be beneficial. He said that OSHA does not have the legal authority to conduct pre-review of applications. Dr. Campbell said that while he opposed a subcommittee doing pre-review of individual applications, he favored a subcommittee to prepare future revisions of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules (i.e., the definitions of Pl-LS, etc.) and to monitor how well the system is working. Dr. Gottesman proposed an amendment to Dr. Krimsky's motion to establish the subcommittee with these functions, but to do no pre-review of individual applications. An amendment to this amendment proposed by Dr. Krimsky to include subcommittee pre-review of individual applications failed by a vote of 3 in favor, 15 opposed, with no abstentions. After further amendments were adopted, the RAC passed the following motion by a vote of 15 in favor, 2 opposed, with 1 abstention:

"A large-scale review subcommittee of the RAC shall be established with the responsibility for advising the RAC on procedures and facilities design pertaining to large-scale operations, and on the performance of local IBCs in reviewing physical containment facilities.

"The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA."

I agree with the establishment of a group to report to the RAC on recommendations for future revisions of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules (i.e., the definitions of P1-LS, etc.) and on the performance of local IBCs in reviewing large-scale physical containment facilities. A Working Group of the RAC was established at the May 1979 meeting to develop the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. These recommendations were reviewed at the December 1979 and March 1980 RAC Meetings before being published in the **Federal Register** on April 11, 1980. Establishing a new "subcommittee" of the RAC to deal with this issue would require a formal change in the RAC Charter. Therefore, I favor reconstitution of the current RAC "Working Group" for this purpose. As noted above (see item IV of this announcement), the RAC has recommended that RAC "members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production." I expect that an individual with these qualifications will shortly be appointed. When this happens, I will ask that the appointee consult with the RAC Chairman and that together they decide on which additional RAC members should be appointed to the reconstituted Large-Scale Review Working Group. The RAC recommended that participation be invited from "NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA." Ideally, all Federal agencies with representatives on the RAC should be invited to participate in the deliberations of the Working Group.

In accordance with the recommendation of the RAC, the Working Group will not be involved in pre-review of individual applications. To assist in its function of advising the RAC on the performance of local IBCs in reviewing physical containment facilities and on future revisions of the Large-Scale Physical Containment Recommendations, the Working Group may request information from individual companies. Since NIH is not a regulatory agency, the intent of any such information collection will be fact-finding, to help in development of general recommendations from the Working Group to the RAC, and not for purposes of regulatory actions directed at individual companies.

In response to recommendations of the RAC, the revised application procedures for large-scale proposals are now promulgated as final, i.e.:

"Application Procedures for Large-Scale Recombinant DNA Experiments.

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The registration document submitted to the local Institutional Biosafety Committee. This should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10–liter limit.

"c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"d. Specification of the P–LS level proposed to be used as defined in the NIH *Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. (Federal Register*, April 11, 1980).

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules and to the extent permitted by law.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

These application procedures are identical to those published for comment on August 21, 1980, except for the elimination of the term "Memorandum of Understanding and Agreement," its replacement by the term "registration document," and addition of the clause "and to the extent permitted by law" in paragraph 5. As discussed above (in item XVI of this announcement), the term "Memorandum of Understanding and Agreement" has been eliminated from the Guidelines. The revised Guidelines, in Section III, describe the required contents of a registration document.

The revised "Application Procedures for Large-Scale Recombinant DNA Experiments" differ from the previous version (adopted at the September 1979 RAC meeting) in requiring that the application specify a "P-LS" level at which the work will be done, rather than requiring individual details of physcial containment. In other respects the Application Procedures adopted at the September 1979 RAC meeting remain identical in the revised Application Procedures promulgated today. As noted in the minutes of the September 6–7, 1979 RAC meeting, review of each large-scale proposal by the RAC, "might be at a meeting, but it also might be through mail ballot." This option will be retained.

Additional Announcements of the Director, NIH

Section IV-E-1-b-(3)-(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Accordingly, several requests for authorization to culture, on a large-scale, recombinant DNA host-vector systems have been received and reviewed by the NIH.

I. Genentech, Inc.

On November 4, 1980, the Director, NIH, on the recommendation of the RAC, approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 host-vector systems containing plasmids into which have been ligated cDNA coding for human leukocyte interferons.

This request was approved with the understanding that Genentech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigators are Drs. Michael Ross and Norm S. C. Lin. The work is to be done at the Pl–LS level of containment at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080.

II. Burns-Biotec Laboratories, Inc.

On November 4, 1980, the Director, NIH, approved requests from Burns-Biotec Laboratories, Inc., a wholly-owned subsidiary of Schering Corporation, for large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte interferon, and certain derivatives thereof, in a 1000 liter fermentor (up to 750 liter working volume) at the Pl–LS level of containment.

The request was approved with the understanding that Burns-Biotec Laboratories, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site. The principal investigator for this project is Dr. Donald E. Baldwin. The large-scale growth of the organisms is to be carried out at plant facilities located in Elkhorn, Nebraska.

Dated: November 14, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because of the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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federal register

Wednesday
September 17, 1980

Part II

Department of Health and Human Services

National Institutes of Health

Program To Assess Risks of Recombinant DNA Research; Proposed First Annual Update

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Program To Assess the Risks of Recombinant DNA Research: Proposed First Annual Update

AGENCY: National Institutes of Health.

ACTION: Notice of proposed first annual update of a program to assess the risks of recombinant DNA research.

SUMMARY: On September 13, 1979 the initial plan was published in the **Federal Register**. This notice sets forth a proposed first annual update of the program to assess the risks of recombinant DNA research. Interested parties are invited to submit comments concerning the plan. After consideration of those comments and comments by the NIH Recombinant DNA Advisory Committee, the Director of the National Institutes of Health will publish the final update in the **Federal Register**.

DATE: Comments must be received by December 16, 1980.

ADDRESS: Written comments and recommendations should be submitted to the Chief, Office of Specialized Research and Facilities, NIAID, Building 31, Room 7A04, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Additional information may be obtained from Dr. John Nutter, Chief, Office of Specialized Research and Facilities, NIAID, National Institutes of Health, Bethesda, Maryland 20205 (301-496-5843).

SUPPLEMENTARY INFORMATION:

I. Introduction

With the issuance in December 1978 of revised guidelines for the conduct of recombinant DNA research, the Secretary DHEW requested that the National Institutes of Health (NIH) prepare an NIH Risk Assessment Plan which after review by the Recombinant DNA Advisory Committee (RAC) and publication in the **Federal Register** for comment, would be made final and updated annually. The present document is the initial response to the request for an annual update.

II. Scientific Aspects

We stated in the Final Plan and it is still our conviction that:

> "The vast majority of information relevant to recombinant DNA risk analysis has already come from research not primarily designed to provide information on risk. This will undoubtedly continue to be the case. This information will be obtained chiefly from publications in the scientific literature, from persons with special scientific knowledge, and from ongoing basic biomedical research. Risk assessment analysis will require continuing review of data developed in the fields of microbiology, infectious diseases, and related biological research.
>
> Some essential information has been, and will continue to be, derived from projects specifically designed to assess various aspects of potential risks associated with recombinant DNA experimentation. Such experiments will be supported by the Intramural and the Extramural programs of NIH. Many experiments may also be conducted in the private sector or may be funded by other agencies or governments.
>
> The essential goal of a successful risk assessment plan will be the development of means to collect, collate, coordinate, evaluate, and disseminate data obtained from all sources."

The Scientific Aspects of the Plan noted that a number of events must occur before a laboratory microorganism becomes a possible risk to people or higher organisms outside the immediate laboratory environment. A major aspect of the risk assessment plan was to acquire and analyze information and data relevant to those elements for the three general categories of host-vector systems in use; prokaryotic, lower eukaryotic and higher eukaryotic systems. Initial emphasis was on the prokaryotic *E. coli* K-12 systems because those were, and remain, the systems predominately used by investigators and because needed areas of investigation had already been identified. Seven areas were identified as requiring particular consideration and progress has been made in collecting and/or analyzing data for all of them. Before considering these it is worth saying that, despite intensive study by the RAC Subcommittee on Risk Assessment and NIH staff, several conferences and workshops to consider specific issues and several experiments, no risks of recombinant DNA research have been identified that are not inherent in the microbiological and biochemical methodology used in such research. A synoptic report of progress follows and all data, reports, and other documents referred to are available on request.

Prokaryotic Host-Vector Systems

1. Survival in the environment and the potential for selective advantage of organisms carrying recombinant DNA should they survive was cited as a matter of concern. NIAID had four contractors working on various aspects of this issue; the contracts were originally awarded to provide independent testing in the process whereby EK2 systems could possibly be elevated to EK3 status. The tests were performed in situations simulating accidential spills in the laboratory, in a model sewage treatment system, in mice and cultures simulating the mouse gastrointestinal system, and finally in germfree mice and humans. The status of the work of all four contractors, at the time the Plan was initially published, can be found in the Recombinant DNA Technical Bulletin, Vol. 2 No. 2, July 1979. The expiration date of all contracts has now been reached and Final Reports are or soon will be available.

a. The work of the contractor testing in "accidental spill situations" was completed shortly after publication of the Plan and was in two areas: (1) to determine whether selected strains of *E. coli*, used as hosts to propagate recombinant-DNA molecules, and some phage and phasmid vectors would survive or retain their capacity to infect or transform host cells after exposure to the environments expected to occur in the event of spill or aerosolization, (2) to inventory and simulate common procedures used in recombinant DNA laboratory studies so that aerosol output potential of the procedures could be accessed.

Considerable effort was expended on quantitative descriptions of the biological characteristics of hosts and vectors of concern in current rDNA studies and the physical disperson aspects have been examined with particular attention to procedures unique to such laboratory work.

The data on biological survival characteristics are applicable to evaluation of potential dosage to workers or to the environments both from accidents creating massive release and from the continuing releases incidental to the laboratory procedures. Evaluation of the effect of survival of the agents in the course of routine work should assist in prudent and cost-effective application of physical and biological containment. Further, it can lend emphasis to the areas in which training in "good practice" can be most productive.

b. The Survival of EK1 and EK2 Systems in Sewage Treatment Plant Models.

In an early series of studies utilizing bench scale models of wastewater treatment facilities, *E. coli* DP50supF, *E. coli* Chi 1776 and phage Charon 4A concentrations were shown to be reduced by at least two orders of magnitude by a conventional treatment chain (including primary settling followed by activated sludge treatment and anaerobic digestion of all sludges generated). This work was described in brief form in the *Recombinant DNA Technical Bulletin* of July 1979.

Similar results are now available for the survival of *E. Coli* Chi 2656 (which carries plasmid pB322) and for *E. Coli* GF 2174 (carrying plasmid pBR325). Evidence also was sought in these studies for transfer of the plasmids to indegenous coliforms and, within the sensitivity of the methods used, such transfer was not demonstrated. Experiments favoring plasmid transfer in raw wastewater and in primary biological sludges also failed to demonstrate such mobilization.

Likewise, *E. Coli* 2e01C carrying a lambda prophage (λcI857 nin5 plac5) was removed effectively by wastewater treatment; no free lambda phage was observed at any point in the treatment train.

Studies have been completed measuring the survival of an indigenous *E. Coli* and *E. Coli* K–12 in these treatment plant models as well. The results of experiments utilizing such genetically tagged organisms confirm an earlier report that conventional domestic wastewater treatment processes will result in at least two log$_{10}$ reductions in concentration of indigenous, EK1, and EK2 organisms. These results are similar to those obtained with the indigenous wastewater microflora. Further reduction of these host-vector populations will depend on appropriate sludge treatment and effluent disinfection.

c. The contractor who was performing tests in mice and in cultures made significant progress during the intervening year. The most notable result was the establishment of a mathematical model to study the exchange of plasmids between normal hosts and indigenous bacteria.

Plasmid transfer in continuous flow (CF) cultures of defined or natural intestinal flora occurred with similar efficiency as in pure cultures in vitro. One can conclude that the capacity of *E. coli* K–12 strains to function as plasmid donors or recipients was not impaired by the presence of an indigenous microflora. *E. coli* Chi 1776 donated plasmid R1drd19 with somewhat lower efficiency than a standard *E. coli* K–12 donor.

Plasmid transfer in mice harboring a defined intestinal microflora appears to have the same degree of efficiency as in the CF cultures, because the rate constants calculated on the basis of the equations are similar to those obtained in CF cultures. However, the validity of this mathematical treatment for mice is not completely certain, because the mathematical model is based on the assumption that the bacteria are freely suspended, which is not likely to be the case in the animal. Nevertheless, the data suggest that plasmid transfer efficiency in the gut should not differ profoundly from that in CF cultures.

These conclusions are based on similarities of transfer rate constants as measures of donor and recipient capacity. The actual rates of transfer are, of course, critically dependent on the concentrations of donors and recipients. The *E. coli* populations in normal mice (and in people) are so low (approx. 10^6 per ml gut content), that little plasmid transfer occurred in the mice after the initial 20 hours of the experiment, i.e. after the large inoculum of donors had passed through the animals. This was true in spite of the fact that the resident *E. coli* were artifically implanted, highly efficient recipients for the highly efficient conjugative plasmid. It is not very common to find *E. coli* of such high recipient capacity in natural gut flora. Despite the fact that the capacities of the *E. coli* strains to donate or receive the plasmid were not seriously impaired in the gut, the quantitative parameters in the normal gut are such that little actual transfer occurred once the large initial inoculum of donors had been eliminated.

From these data one may tentatively conclude that the probability for triparental transfer of a non-cojugative plasmid in the normal gut would be exceedingly small. The only circumstances in which one could imagine a realistic possibility for triparental transfer to occur, would be at times when abnormally high *E. coli* populations are present in the gut, as may be the case during fasting and other types of stress, in diarrhea or as a consequence of antibiotic therapy.

d. The major effort of the contractor who was testing in both mice and humans in the interim has been to initiate triparental mating studies using Chi 1666 (an EK1 host), the plasmid pBR322 and two additional mobilizing plasmids.

The studies are designed to determine if the presence of a mobilizing plasmid in a bacterial host cell can cause the transfer of the non-conjugative pBR322 to the indigenous microflora of either mice or humans.

Two studies have been done in mice. In both cases germ-free mice were colonized with human *E. coli* to provide an array of potential recipients and then fed the Chi 1666 containing the three plasmids. In the first study 5×10^9 organisms were fed once and fecal samples from the mice were pooled to facilitate assay; Chi 1666 could only be isolated during the first day of the study. In the second mouse study, the Chi 1666 containing the plasmids were fed daily for 4 days to the mice and their drinking water contained tetracycline. In this latter group the Chi 1666 survived at levels of 10^3/gram or less for the 4 days. In neither case, however, was the pBR322 mobilized to the indigenous flora.

Finally, Chi 1666 containing pBR322 and the two mobilizing plasmids was fed to 4 human subjects. More than 70 fecal samples were collected during the study and a large number of cultures and subcultures (2500) performed. The Chi 1666 survived from 3½ to 7 days and that interval corresponds well with previous data from studies in which Chi 1666 was fed to humans with the without pBR322. While the data for transfer of pBR322 await DNA:DNA colony hybridization and further confirmation, from the data already available on the two transferable plasmids, we can make certain estimates. Highly transferable pSL222-4 was found to transfer to coliforms at a frequency no higher than 8×10^{-6}. This value is 10^{-6} lower than seen when the plasmid is tested under the best laboratory conditions. Thus pBR322, which is transferable by F-like R plasmids at 10^{-4}–10^{-6} would be expected to be transferred at an extremely low frequency; probably below experimentally detectable levels. The same is presumably true of potential mobilization by the second conjugative plasmid, although the data for their mobilization of pBR322 are not readily available. We would estimate that pBR322 would be transferred at a frequency at least 10^4–10^6 fold less than the conjugatable plasmid, i.e., 10^{-8}–10^{-11}.

2. Progress on the evaluation of the transmission of vectors from *E. coli* K-12 to other bacteria in the gastrointestinal tract of animals and human beings has been made via three approaches.

a. Data reported in section 1 by two of the contractors relates directly to this question.

b. A meeting was convened on August 30, 1979 at the NIH with the purpose of considering Falmouth Workshop Protocols I and II (Jour. Infect. Dis. *137*, 704–706, 1978) and some related issues. Dr. Stanley Falkow served as Chairman and sixteen participants and NIH staff attended.

Protocol I addressed the colonization and transmission of plasmids from *E. coli* K–12 in the gastrointestinal tract of humans to other bacterial strains in the intestinal flora. The Working Group unanimously recommended that the NIAID not initiate new studies to pursue the investigations as written in Protocol I. This judgment was based on a review of data that existed at the time of the Falmouth Workshop, a consideration of some newer published data and the results of contracts that NIAID was supporting. As written by the Falmouth Workshop Participants, the experiments were to be based on *E. coli* K–12 and this was judged to not be a fruitful experimental model. It was a clear consensus of the Group that, based on the available data, it can be predicted that only negative results will be obtained and that limited resources could be better expended in other pursuits.

Protocol II was designed to study the transmission of plasmids from *E. coli* K–12, including Chi 1776, into the normal intestinal flora utilizing a germ-free mouse model. The Working Group unanimously supported the view that the NIAID should not initiate new studies for the Protocol as it was written but to rely on the contracts to supply some additional data. This judgment was based on the same reasoning and data base considered for Protocol I.

During discussion of the issues cited above it was obvious that the Working Group felt that a more beneficial use of monies would be to support the training of workers in good microbiological laboratory practices and to support research aimed at gaining a better basic scientific understanding of bacterial colonization and plasmid mobilization. Toward this last goal the Working Group strongly recommended that the NIAID support studies that could obtain quantitative data, expand scientific knowledge in an important area and which may prove useful at some future date for risk assessment. The Group felt that such studies should be performed directly in humans and employ wild type *E. coli* (not K–12). Strain HS containing pBR325 was suggested as a good initial combination and the study should be developed to assay for both survival and transfer to the indigenous flora. [These studies are now in their early stages and being performed by an NIAID contractor at the University of Maryland Medical School.]

The full transcript of this meeting and a verbal report was made to RAC at their March 1980 meeting and the recommendations were approved by that Committee.

c. NIAID identified two relevant grant applications and the National Advisory Allergy and Infectious Diseases Council supported selective payment of these projects for inclusion into the risk assessment program.

One grantee will focus on the mechanisms that control human and animal gut flora. Of the four stated proposed aims of the research plan three relate to issues of importance to the NIH Recombinant DNA Risk Assessment Program. They are: (1) characterize and extend the application of anaerobic continuous flow cultures, (2) analyze the efficiency of plasmid and bacteriophage transfer, and (3) determine whether human microflora can be maintained in gnotobiotic mice and in anaerobic continuous flow cultures. The issue of mobilization of vector plasmids to the indigenous flora has always been a concern when considering the use of *E. coli* K–12 based host-vector systems. Most recently concern has been expressed over the potential for exchange of plasmids between the Enterobacteriaceae and the anaerobic flora, principally members of the genus Bacteroides. This project has the capacity for filling a significant void in experimental data.

A second grantee will be exploring a related issue which focuses on the molecular mechanisms of *E. coli* colonization of the intestine, specifically on the relative importance of plasmid or chromosomal determinants of colonization.

At this point in time the majority of experiments using recombinant DNA technology employ host-vector systems based on *E. coli* K–12 and its plasmids or bacteriophages. Prominent among the scenarios raised early in the debate over use of this technology was the possible colonization of the intestinal tract by host-vector systems followed by various consequences depending on the elaboration of a product which would cause harm to the individual by either direct or indirect mechanisms. There now are a considerable number of studies describing the survival of various types of *E. coli* in the intestinal tracts of man and mice and they demonstrate a tremendous disparity in the survivability and colonization potential of such strains. A complete understanding of those factors that control survival and colonization may permit the development of both safer and more useful *E. coli* hosts in the future as well as perhaps provide data suggesting adjustments in the physical containment requirements of the NIH Guidelines governing use of this technology.

3. Experiments testing *E. coli* K–12 host-vector systems carrying recombinant DNA for virulence have been done by NIH scientists. The studies were designed to determine the pathogenicity and stability of shotgun clones of Saccharomyces DNA when used with both plasmid and bacteriophage vectors. In this experimental model, which utilized mice, there was no evidence that the presence of segments of the entire yeast genome altered the inherently low pathogenicity of *E. coli* K–12 in any way.

4. A Workshop was convened to consider two areas [within the NIH Program] recommended by the Risk Assessment Subcommittee of RAC. This Workshop was designed to define the scientific issues and assess the potential risks of: (a) Possible direct adverse effects of hormone-producing strains of *E. coli* K–12, and (b) the possible occurrence of autoantibodies or autoreactive cells due to the production of eukaryotic polypeptides (including hormones) by *E. coli* K–12 should they colonize higher organisms.

In the charge to the Workshop it was noted that these potential risks were under consideration because there is still debate over the degree of possible risk, even though *E. coli* K–12 has apparently lost those known characteristics that are required for colonization of the normal intestinal tract.

The purpose was to decide whether the two specific possibilities are valid scientific hypotheses if such an event did occur; and if valid, to determine if sufficient experimental data already exists to make a final judgment concerning possible risk. If data were insufficient, the participants were asked to outline those types of studies necessary to develop the definitive information on these issues.

The meeting brought together 92 scientists from the fields of immunology, endocrinology, physiology, microbiology, infectious diseases and other appropriate disciplines. The Chairmen's Summary and a full transcript of the Final Plenary Session was distributed to all participants and also the RAC for their review at the June 1980 meeting. In addition to the report of the Director, NIAID, Drs. Setlow and Campbell prepared a separate report and the RAC Risk Assessment Subcommittee reported their analysis of the Workshop.

NIAID Plans in implementing the four prime recommendations was influenced by the RAC discussions. At this time we propose to respond to the recommendations as follows:

a. It was recommended that experiments of the folowing general type should be performed: "*E. coli* cells carrying recombinant NDA and making a mammalian protein are introduced into living animals under conditions where they cause genuine infection (e.g.

an abscess), with subsequent monitoring for breakdown of immune tolerance."

This subject stimulated the most discussion during the final plenary session of the Workshop. The sentiment was to acquire the data as a matter of scientific interest rather than to answer the risk assessment question. An RFP is being developed to solicit applications from interested investigators. Immunologists from the NIAID staff will design the experimental protocol for the workscope and other specifications of the study which is most likely to be an animal model system employing insulin. The matter however is still in the formative stage and proposers will have considerable leeway in outlinging plans to answer the primary questions.

b. Additional information was requested on the handling and absorption of polypeptides in normal and pathologic colon and the potential effects of synthetic peptides on the bowel itself.

We have confirmed that this is an issue about which there are little direct data. The Workshop participants opined that although their own calculations revealed that only extremely small amounts of hormones would be produced under the most ideal conditions, data should still be sought for potential future needs. The issue of the potential effects of synthetic peptides on the bowel itself is different from that of hormones and that discussion was centered on interferon and its direct effect on cells.

Although this will be a difficult study to initiate because of the technical difficulties as well as the process for approval of research in human subjects, NIAID is considering solicitation of grant proposals through an RFA. The study will have as its objective a determination of the fate of peptide hormones when deposited in the distal small intestine and large intestine of humans. These sites are relevant to the production of hormones by recombinant DNA technology because they represent the regions of colonization by *E. coli.*

c. It was suggested that additional information is needed on the potential transfer of plasmids to anaerobic bacteria from *E coli.* This recommendations differs from the findings of the ad hoc group convened in August 1979 which RAC reviewed at the March 1980 meeting. That expert Group noted that by studing the epidemiology of plasmids the *conjugative plasmids of E. coli* are not found within the anaerobic flora of the gut. The uniform use of non-conjugative plasmids in rDNA research further reduces the likelihood of transfer.

It is likely that the requested data will be forthcoming from NIH (NIAID) supported studies on plasmids through the regular grant-supported research programs and that new initiatives are not needed. In fact, the NIAID Advisory Council identified a grant application for selective payment that addressed some aspects of this issue; this action will initiate the acquistion of the data at an early date. There is no plan to develop a RFA at this time but NIAID staff will continue to identify all appropriate incoming applications as potential sources of data.

d. It was recommended that NIH communicate with the Center for Disease Control (CDC) about possible types of health surveillance for workers using recombinant DNA.

A CDC representative was present at the Pasadena meeting and discussed some approaches to this issue. Furthermore, representatives of the National Institute for Occupational Safety and Health and the Occupational Safety and Health Administration were also present and therefore, are cognizant of the Workshop recommendation. All of these agencies are members of The Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research which is discussing this issue.

5. NIH scientists have continued a further evaluation of the biological activity of polyoma virus DNA cloned in *E. coli* host vector systems. It had been reported (Science *203:* 883–892, 1979), when the Final Plan was initially published that polyoma DNA was noninfectious when cloned in EK2 plasmid and bacteriophage host-vector systems.

As a futher step in evaluating the biologic activity of the polyoma-plasmid and polyoma-lambda recombinant DNA host-vector systems, the scientist tested the ability of the EK2 systems containing polyoma DNA to induce tumors in suckling hamsters.

These animals are highly sensitive to tumor induction by polyoma virus and in some cases even subgenomic fragments of viral DNA can induce tumors in them. The results (Science *205;* 1140–1142, 1979) indicated that inoculation of suckling hamsters with 2×10^8 live cells of *E. coli* K–12 strain Chi 1776 carrying the complete genome of polyoma virus in a recombinant plasmid, failed to induce tumors in any of 32 recipients. Also, lambda phage DNA and particles with a monomeric insert of polyoma DNA did not induce tumors. Purified recombinant plasmid DNA, as well as phage particles and DNA containing a head-to-tail dimer of polyoma DNA, showed a low degree of oncogenicity, comparable to that of polyoma DNA prepared from mouse cells. These findings support the previous conclusions, based on infectivity assays in mice, that propagation of polyoma virus DNA as a component of recombinant DNA molecules in *E. coli* K–12 reduces its biologic activity many orders of magnitude relative to the virus itself.

Currently both the infectivity and tumorigenicity experiments are being extended. In one, *E. coli* K–12 (EK1) containing recombinant plasmids consisting of two copies of polyoma virus DNA and one copy of pBR322 plasmid DNA are being inoculated into newborn hamsters and weanling mice. It is also planned to test lambdaphage lysogens (*E. coli* K–12 containing one or two copies of a lambda bacteriophage-polyoma virus DNA recombinant integrated into the bacterial chromosome) by inoculation into appropriate animal model systems. Results will be published in the open scientific literature and will be presented in a subsequent update of the Final Plan.

6. NIH scientists are also determining the biological activity of *E. coli* K–12 clones carrying DNA copies of an RNA tumor virus. These studies involve the administration of bacterial preparations containing Harvey sarcoma virus DNA to hamsters. These studies are still in progress and when definitive results have been obtained they will be published in the open scientific literature and will appear in an annual update of the final plan.

7. Although they had not appeared in the previous Final Plan, two additional NIAID contract-supported projects are peripherally related to the total risk assessment activities.

a. RAC had suggested that a model cloned DNA segment be constructed to serve as a uniform heterologous DNA segment for risk assessment studies. This endeavor has been successfully completed by an investigator at the University of Wisconsin and employed, as a source of DNA, the plasmid NR79. The EcoRI fragment NR79–R1–G1 contains genes encoding for chloramphenicol, kanamycin and, sulfonamide resistance, as well as the promoters that govern these resistances. NR79–R1–G1 was shown not to have an internal EcoRI site and also not to be capable of replicating by itself or with the help of other plasmids. Experimental results indicated that NR79–R1–G1 is not capable of autonomous replication and does not contain an origin of replication that can function in a recombinant plasmid. The antibiotic resistances encoded for by NR79–R1–G1 were tested

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and found not to be transposable, that is gene movement and insertion at a new site by a mechanism which does not involve the homologous recombination system of the host strain.

Since the fragment is not capable of autonomous replication, plasmid pJT353, a recombinant plasmid containing NR79-R1-G1 cloned into the EcoRI site of pBR322, is the suggested way of propagating this fragment. Stocks of *E. coli* strain KH802 containing pJT353 have been preserved for the propagation and isolation of NR79-R1-G1. Protocols used for the isolation of purified plasmid pJT353 (NR79-R1-G1 and pBR322) and purified NR79-R1-G1 fragment are available.

b. A contract has been awarded to the University of Minnesota to develop a Comprehensive Course on Microbiological Principles and Techniques for Work with Potentially Biohazardous Agents Including Recombinant DNA. The Principal Investigator will work with the Board of Education and Training of the American Society for Microbiology (ASM) in developing the materials.

In 1977, the ASM undertook a study to devise standards of training for recombinant DNA research workers. A proper state of training of the laboratory workers is the first line of containment and it was their opinion that such training standards should be set by knowledgeable professionals. The product of that study was not standards but rather an outline of a body of knowledge which it was felt that any Principal Investigator should be aware of before independently embarking on recombinant DNA research. This contract will develop all necessary resources to present a course based on the ASM findings.

The plan is for the NIAID to support the development of the resource materials and once that phase is completed, the NIH Division of Safety will have the materials reproduced and distributed to the various Institutions where the NIH supports research to assist them in performing their own local training responsibilities. We will devise both a standard lecture/laboratory course and self study aides.

Eukaryotic Host-Vector Systems

Lower eukaryotic and higher eukaryotic host-vector systems were given a lower priority in the Final Plan than prokaryotic systems.

After discussion at both the March and June 1980 meetings, the RAC recommended modifying the Guidelines to include *Saccharomyces cerevisiae* host-vector systems under section III-O. During their consideration the RAC considered information that: (1) *S. cerevisiae* is nonpathogenic, (2) it does not implant in the intestine, (3) the dilute conditions in which it is found in nature are extremely unfavorable for mating, (4) it does not efficiently compete with wild strains of *S. cerevisiae*, (5) it is fully sensitive to autoclaving and disinfection by standard agents, and (6) mammalian genes cloned in *S. cerevisiae* are not expressed.

RAC also considered the question of proper physical containment for higher eukaryotic viral vectors. The Guidelines have been modified to permit work at more relaxed levels of physical containment that previously required.

The Guidelines have been modified to permit recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) to be propagated and maintained in cells in tissue culture using P1 containment.

It must be shown that the cells lack helper virus for the specific families of the defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one family but each fragment must be less than two thirds of a genome.

No initiatives have been started to directly answer the issues raised in the Final Plan relative to eukaryotic host-vector systems. NIH will continue to monitor the results of free ranging research to collect useful data that can be analyzed as part of the risk assessment process.

III. Implementation

The Final Plan stated that NIAID would recruit and appoint an eminent scientist as a Special Assistant to the Director, NIAID for Risk Assessment. Attempts to recruit a person to fill this position were made including national advertising. No individual with the desired level of credentials and broad recognition by the various scientific disciplines was identified who was willing to undertake this work at the NIH under the conditions we could offer. By mid-year it had become evident that the probable scope and number of various activities encompassed by the Risk Assessment Plan would probably stabilize at the current level and not increase further. Consequently the necessity for a Special Assistant was reconsidered and alternatives for satisfying the needs were evaluated. A decision was made to distribute the proposed functions of the Special Assistant to the Office of Recombinant DNA Activities and the Office of Specialized Research and Facilities, both within the NIAID. When appropriate these offices will use ad hoc consultants in fulfilling the tasks originally described for the Special Assistant and for which staff and the RAC Risk Assessment Subcommittee feel that additional or specific expertise is required.

The remainder of the Implementation section of the plan remains in effect as stated. Most implementation actions have been cited earlier in this document, however, an important part of the Plan are periodic reports to the RAC. In this regard, the Director, NIAID reports to the RAC at each meeting on activities related to risk assessment and provides thereby an important and continuing review of progress and receives the advice or comment of this advisory group.

Dated: September 8, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*.

Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A-95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that Circular.

[FR Doc. 80-28453 Filed 9-16-80; 8:45 am]
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Institutional Biosafety Committee Chairpersons; Meeting

Notice is hereby given of a Workshop for the Institutional Biosafety Committee (IBC) Chairpersons. The Workshop is being held on November 24 and 25, 1980, at the Shoreham Hotel on Calvert St., Washington, D.C. and is being sponsored by the National Institute of Allergy and Infectious Diseases. The meeting will convene at 9:00 a.m. on November 24, 1980 and will be open to the public; attendance will be limited to space available.

The Workshop is designed to provide an opportunity for the Chairpersons of all Institutional Biosafety Committees to study together how best to address and resolve some problems they may face and to serve as a stimulus for action at the local level. In addition, the Workshop will serve as the first stage of a formal evaluation process to assess whether the IBCs as constituted have been able to meet the increasing responsibilities that changes in the NIH Guidelines for Recombinant DNA Research have thrust upon them and to identify and seek solutions to problems at both the federal and institutional level.

Topics covered during the meeting in plenary sessions will include:

- Review and Update of the NIH Guidelines for Recombinant DNA Research.
- Recombinant DNA Advisory Committee Procedures and Operations.
- Operations of Institutional Biosafety Committees (IBC).
- Other Federal Regulations and Guidelines that Impact on Biomedical Science.
- Proposed plan for evaluation of the IBCs.

In addition to the plenary sessions there will be three workshops:

- The IBC as a Means of Implementing Institutional Oversight.
- Health Surveillance, Monitoring and Certification.
- IBC Procedures and Operations.

Further detailed information can be obtained from Dr. John Nutter, Chief, Office of Specialized Research & Facilities, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, (301-496-6752).

Dated: October 28, 1980.

Suzanne L. Fremeau,

Committee Management Officer, NIH.

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Note.—NIH programs are not covered by OMB Circular A-95 because they fit the description of "programs not considered appropriate" in Section 8(b) (4) and (5) of that Circular.

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INSTITUTIONAL BIOSAFETY CHAIRPERSON'S MEETING

Sponsored By:

THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
November 24-25, 1980
Shoreham Hotel
Calvert St., Washington, D.C.

AGENDA

Sunday, November 23

6:00 - 10:00 p.m.	Registration	(Palladian Foyer)

Monday, November 24

7:30	Breakfast	(Blue Room)
7:00 - 9:00 a.m.	Registration	(Palladian Foyer)
9:00 - 10:30 a.m.	Plenary Session I	(Palladian Room)

MODERATOR: DR. RICHARD KRAUSE, DIRECTOR, NIAID

	Opening Address	Dr. Donald Fredrickson Director, NIH
	Objectives of Conference	Dr. Richard Krause Director, NIAID
	Review and Update of Guidelines	Dr. William Gartland Director, ORDA, NIAID
	RAC Procedures and Operations	Ray Thornton, J.D. Chairperson, RAC
10:30 - 11:00 a.m.	Coffee Break	

11:00 a.m. - 12:30	Plenary Session II (Palladian Room)	
	Panel on Operations of IBC's	
	The IBC as a Means of Implementing Institutional Oversight	Dr. Patrick Gage Hoffman-La Roche Inc.
	The IBC from the Vantage Point of a Community Member	Robert Spanner, J.D. Community Member, University of Californi San Francisco, CA
	IBC Procedures and Operations	Dr. Alan Garber Baylor College of Medicine
	Health Surveillance, Monitoring and Certification	Dr. Melvin H. Chalfen Massachusetts Institute of Technology
12:30 - 2:00 p.m.	Lunch (Blue Room)	
2:00 - 5:00 p.m.	Workshops	

There will be 3 workshops occurring simultaneously in separate rooms. The procedure to be used is the following (See attachment for details):

2:00 - 3:20 p.m.	First Session Workshops A, B, & C
3:20 - 3:40 p.m.	Break
3:40 - 5:00 p.m.	Second Session Workshops A, B, & C
5:00 - 6:30 p.m.	Cash Bar Reception (Ambassador Room)

Tuesday, November 25

7:30 a.m.	Breakfast (Blue Room)	
9:00 - 10:30 a.m.	Plenary Session III (Palladian Room) Report on Workshops	
MODERATOR:	DR. WILLIAM J. GARTLAND - DIRECTOR, ORDA, NIAID	
	The IBC as a Means of Implementing Institutional Oversight	Ms. Patricia King, RAC
	Health Surveillance, Monitoring and Certification	Dr. James O. Mason, RAC
	Procedures and Operations	Dr. Sheldon Krimsky, RAC
10:30 - 11:00 a.m.	Coffee Break	
11:00 - 12:00	Proposed Evaluation Plan (Palladian Room)	
	Presentation by NIAID Staff	Dr. Marilyn Bach, NIAID
	Comments by IBC Chairpersons	Dr. Edward A. Adelberg Yale University Dr. Rose M. Litman University of Colorado
12:00 - 1:30 p.m.	Lunch	

1:30 - 4:00 p.m. Plenary Session IV (Palladian Room)

An Overview of the Other Federal Health and Environmental Regulations and Guidelines That Impact on the Biomedical Research Community

MODERATOR: DR. W. EMMETT BARKLEY, DIRECTOR, DIVISION OF SAFETY, NIH

Proposed Biosafety Guidelines for Microbiological and Biomedical Laboratories	Dr. John Richardson, Dir. Office of Biosafety Center for Disease Control Department of Health and Human Services
Draft Guidelines for the Laboratory Use of Chemical Carcinogens	Dr. W. Emmett Barkley Director, Division of Safety, OD, NIH
Occupational Health Standards and Their Application to the Biomedical Research Laboratory	Dr. Bailus Walker, Dir. Health Standards Program Occupational Safety and Health Administration Department of Labor
Federal Regulations Pertaining to the Management and Disposal of Toxic Chemical and Biological Wastes Generated by the Biomedical Research Laboratory	Dr. Jack Lehman, Dir. Hazardous & Industrial Waste Division Environmental Protection Agency
Low Level Radioactive Waste Management - Federal Requirements & Guidelines	Dr. Richard E. Cunningham Director, Div. of Fuel Cycle and Material Safety Nuclear Regulatory Commission

ATTACHMENT

The workshop topics will be:

WORKSHOP A: (Palladian Room)

The IBC As a Means of Implementing Institutional Oversight

Workshop Team:	Ms. Patricia A. King, RAC Member Dr. Samuel Boyer, The Johns Hopkins University Dr. Bernard Talbot, NIH

Will include topics such as:

1) How institutional and non-institutional members are selected and whether remunerated
2) Role, value, function of various members based on professional expertise
3) Scope of responsibilities of committee as a whole and of individual committee members
4) Making and implementing difficult decisions, and the impact of difficult decisions--internal politics and pressures

WORKSHOP B: (Executive Room)

Health Surveillance, Monitoring and Certification

Workshop Team:	Dr. James O. Mason, RAC Member Dr. Gerald J. Spahn, The Salk Institute Dr. Stanley Barban, NIH

Will include topics such as:

1) How is medical surveillance provision interpreted and implemented: by whom and for whom; blood samples, physical exams, cultures
2) Accident reporting and investigation of infections
3) Monitoring laboratory facilities and operations; how is the laboratory certified
4) Interaction with PI after approval.

WORKSHOP C: (Board Room)

Procedures and Operations

Workshop Team:	Dr. Sheldon Krimsky, RAC Member Dr. Gary A. Wilson, Miles Laboratories, Inc. Dr. Elizabeth Milewski, NIH

Will include topics such as:

1) Administrative and technical support

2) Streamlining and excessive bureaucracy

3) Recordkeeping and availability of records, minutes, etc. and keeping track of modifications in the guidelines

4) Types of training at different levels
IBC Chairpersons/members/PI/Technician/dishwasher

11/14/79

DRAFT

PART VII

PHYSICAL CONTAINMENT GUIDELINES FOR LARGE-SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

VII. PHYSICAL CONTAINMENT FOR LARGE-SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

This part of the NIH Guidelines specifies the laboratory practices, containment equipment and special facility design for large-scale (greater than 10 liters of culture) research and production involving organisms containing recombinant DNA molecules. It shall apply to all large-scale research and production activities approved by the Director, NIH in accordance with Sections IV-E-1-b-(1)-(e) and IV-E-1-b-(3)-(d) of the NIH Guidelines.

All provisions of the NIH Guidelines shall apply to large-scale research and production activities with the following modifications:

- ° Part VII shall replace Section II-B when quantities in excess of 10 liters of culture are involved in research or production.
- ° The institution shall appoint a biological safety officer if it engages in large-scale research or production activities involving organisms containing recombinant DNA molecules. The duties of the biological safety officer shall include those specified in Section IV-D-4.

VII-A. Selection of Physical Containment Levels. The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the physical containment levels established in Sections II and III of these guidelines. For purposes of large-scale research or production, two physical containment levels are established. These are referred to as P2-LS and P3-LS.

VII-A-1. Physical Containment Level For P1 and P2 Experiments. The P2-LS level of physical containment is required for large-scale research or production of organisms requiring either P1 or P2 containment at the laboratory scale.

VII-A-2. Physical Containment Level For P3 Experiments. The P3-LS level of physical containment is required for large-scale research or production of organisms requiring P3 containment at the laboratory scale.

VII-A-3. Physical Containment Level For P4 Experiments. No provisions are made for large-scale research or production requiring P4 containment. If necessary, these requirements will be established on an individual case basis.

VII-B. P2-LS Level.

VII-B-1. Laboratory Practices.

VII-B-1-a. Only persons who have a need and who have been advised of the nature of the work shall enter the production area.

VII-B-1-b. The universal biohazard sign shall be posted on all equipment used to process or store organisms containing recombinant DNA molecules, and on all access doors to the production area during the period when any work involving the organisms is in progress. This includes periods when equipment contaminated with the organisms is undergoing sterilization.

VII-B-1-c. The production area shall be kept neat and clean.

VII-b-1-d. Eating, drinking, smoking, and the storage of food are prohibited in the production area.

VII-b-1-e. An insect and rodent control program shall be maintained.

VII-B-1-f. Animals shall not be permitted in the production area.

VII-B-1-g. The wearing of protective garments or uniforms is required. Such clothing shall not be worn outside of the production area. Clothing shall be decontaminated before laundering.

VII-B-1-h. Access doors to laboratories and rooms housing production operations shall be kept closed while work is in progress.

VII-B-1-i. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

VII-B-1-j. Precautions shall be taken in the conduct of all procedures to minimize aerosol formation.

VII-B-1-k. Use of hypodermic needles shall be avoided when alternative methods are available.

VII-B-1-l. Bulk volumes or concentrates of inactivated organisms containing recombinant DNA molecules shall be contained in durable leak proof containers for transport outside the production area. Such materials may be transferred to other facilities provided that inactivation of the organisms has been demonstrated.

VII-B-1-m. All contaminated wastes shall either be steam-sterilized (autoclaved) before disposal or incinerated. Contaminated equipment and materials shall be steam-sterilized or disinfected prior to processing for reuse.

VII-B-1-n. Wastes and contaminated equipment or materials that are to be steam-sterilized, disinfected, or incinerated at a location outside the production area shall be transported in a durable leak proof container which is sealed prior to exit from the production area.

VII-B-1-o. All work surfaces shall be disinfected following completion of procedures involving the handling of organisms containing recombinant DNA molecules.

VII-B-1-p. Persons shall wash their hands after completing procedures with organisms containing recombinant DNA molecules, following removal of protective gloves or clothing, and when leaving the production area.

VII-B-1-q. Persons working in the production area shall be trained (as required) in the operation of the equipment. All persons shall be trained in emergency procedures.

VII-B-1-r. Equipment and materials required for the management of accidents involving organisms containing recombinant DNA molecules shall be available in the production area.

VII-B-1-s. The production area shall be decontaminated in accordance with established procedures following spills or other accidental release of organisms containing recombinant DNA molecules.

VII-B-2. Containment Equipment.

VII-B-2-a. Biological safety cabinets or other containment units shall be used for all equipment or manipulations that produce aerosols where these procedures involve organisms containing recombinant DNA molecules. The exhaust from the containment equipment shall be fitted with a HEPA filter.

VII-B-2-b. Equipment used in the production or processing of organisms containing recombinant DNA molecules shall be designed, fabricated, and installed in a manner which provides for operation and maintenance for its

its intended purpose. The equipment shall:

VII-B-2-b-(1). Be designed, fabricated, and installed in a manner which provides for the containment of materials used in, or resulting from, the propagation of organisms containing recombinant DNA molecules. Containment refers to the prevention of the release of materials to the environment during periods when the unit contains viable organisms.

VII-B-2-b-(2). Be designed, fabricated, and installed in a manner which facilitates observation during operation to assure the integrity of containment.

VII-B-2-b-(3). Be designed and fabricated in a manner which provides for sterilization of surfaces exposed to organisms containing recombinant DNA molecules prior to exposure of these surfaces to the environment.

VII-B-2-b-(4). Be designed, fabricated, and installed in a manner which facilitates disassembly, cleaning, and maintenance to assure integrity of the containment features of the unit.

VII-B-2-b-(5). Have all connected utility services protected against contamination.

VII-B-2-b-(6). Be tested for integrity of the containment features using a non-debilitated strain of the species of the host organism to be employed in production. Testing shall be accomplished prior to the initial production of organisms containing recombinant DNA molecules, and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism.

VII-B-2-b-(7). Be permanently identified. This identification shall be used in all records reflecting testing, operation and maintenance and in all documentation relating to use of this equipment for the production of organisms containing recombinant DNA molecules.

VII-B-3. Special Facility Design.

VII-B-3-a. An autoclave for sterilization of wastes and contaminated materials shall be available in the same building used for the production of organisms containing recombinant DNA molecules.

VII-B-3-b. Equipment used for the propagation, inactivation, or other processing of volumes greater than 10 liters of organisms containing recombinant DNA molecules shall be isolated by a curb or similar architectural feature of such size as to contain the total liquid volume contained in the equipment.

VII-B-3-c. Floor drains serving a curbed or similar area shall be sealed during periods when production is in progress.

VII-B-3-d. The floor shall be readily cleanable and penetrations through the floor shall be sealed to preclude passage of liquids.

VII-C. P3-LS Level.

VII-C-1. Laboratory Practices.

*VII-C-1-a. Personnel entry into the production area shall be through an air lock, or other controlled access area.

*VII-C-1-b. Persons entering the production area shall exchange their personal clothing for work garments such as jumpsuits, or pants and shirts, plus head cover, and shoes. On exit from the production area the protective clothing may be stored in a locker separate from that used for personal

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*Not required for P2-LS.

clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

*VII-C-1-c. Entry into the production area during periods when productio is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry all persons shall be advised of the nature of the work conducted and shall be fully informed of the operating practices and emergency procedures.

*VII-C-1-d. Persons under 18 years of age shall not be permitted to enter the production facility.

*VII-C-1-e. The universal biohazard sign shall be posted on all equipment used to process or store organisms containing recombinant DNA molecules, and on controlled access doors and all internal doors when any work involving the organisms is in progress. This includes periods when equipment contaminated with the organisms is undergoing sterilization.

VII-C-1-f. The production area shall be kept neat and clean.

VII-C-1-g. Eating, drinking, smoking and storage of food are prohibited in the production area.

*VII-C-1-h. Animals and plants shall be excluded from the production area.

VII-C-1-i. An insect and rodent control program shall be maintained.

*VII-C-1-j. Persons shall wear protective gloves when conducting procedures with organisms containing recombinant DNA molecules. Immediately on completion of the procedure the gloves shall be aseptically removed and decontaminated.

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*Not required for P2-LS.

VII-C-1-k. Access doors to laboratories and rooms housing production operations shall be kept closed while work is in progress.

VII-C-1-l. Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

*VII-C-1-m. Procedures which may result in the release of aerosols shall be avoided or properly contained to prevent exposure of persons and contamination of the environment.

VII-C-1-n. Use of hypodermic needles shall be avoided when alternative methods are available.

VII-C-1-o. Bulk volumes or concentrates of inactivated organisms containing recombinant DNA molecules shall be contained in durable leak proof containers for transport outside the production area. Such materials may be transferred to other facilities provided that inactivation of the organisms has been demonstrated.

*VII-C-1-p. All contaminated wastes shall either be steam-sterilized (autoclaved) within the production area before disposal or incinerated. Contaminated equipment and materials shall be steam-sterilized or disinfected within the production area prior to processing for reuse.

VII-C-1-q. Contaminated wastes to be disposed of by incineration shall be placed in a leak proof container which is sealed prior to exit from the production area.

VII-C-1-r. All work surfaces shall be disinfected following completion of procedures involving the handling of organisms containing recombinant DNA molecules.

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*Not required for P2-LS.

VII-C-1-s. Persons shall wash their hands following removal of protective gloves or clothing and when leaving the production area.

VII-C-1-t. Persons working in the production area shall be trained (as required) in the operation of the equipment. All persons shall be trained in emergency procedures.

VII-C-1-u. Equipment and materials required for the management of accidents involving organisms containing recombinant DNA molecules shall be available in the production area.

VII-C-1-v. The production area shall be decontaminated in accordance with established procedures following spills or other accidental release of organisms containing recombinant DNA molecules.

VII-C-2. Containment Equipment.

VII-C-2-a. Biological safety cabinets or other containment units shall be used for all equipment or manipulations that produce aerosols where these procedures involve organisms containing recombinant DNA molecules. The exhaust from the containment equipment shall be fitted with a HEPA filter.

VII-C-2-b. Equipment used in the production or processing of organisms containing recombinant DNA molecules shall be designed, fabricated, and installed in a manner which provides for operation and maintenance for its intended purpose. The equipment shall:

VII-C-2-b-(1). Be designed, fabricated, and installed in a manner which provides for the containment of materials used in, or resulting from, the propagation of organisms containing recombinant DNA molecules. Containment refers to the prevention of the release of materials to the environment during periods when the unit contains viable organisms.

VII-C-2-b-(2). Be designed, fabricated, and installed in a manner which facilitates observation during operation to assure the integrity of containment.

VII-C-2-b-(3). Be designed and fabricated in a manner which provides for sterilization of surfaces exposed to organisms containing recombinant DNA molecules prior to exposure of these surfaces to the environment.

VII-C-2-b-(4). Be designed, fabricated, and installed in a manner which facilitates disassembly, cleaning, and maintenance to assure integrity of the containment features of the unit.

VII-C-2-b-(5). Have all connected utility services protected against contamination.

VII-C-2-b-(6). Be tested for integrity of the containment features using a non-debilitated strain of the species of the host organisms to be employed in production. Testing shall be accomplished prior to the initial production of organisms containing recombinant DNA molecules, and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organisms.

VII-C-2-b-(7). Be permanently identified. This identification shall be used in all records reflecting testing, operation and maintenance and in all documentation relating to use of this equipment for the production of organisms containing recombinant DNA molecules.

VII-C-3. Special Facility Design.

*VII-C-3-a. The production area shall have a separate entry area for controlled access. The entry area shall be a double doored space such as an air lock, anteroom or change room that separates the production area from the balance of the facility.

*VII-C-3-b. The surfaces of walls, ceilings, and floors in the production area shall be such as to permit ready cleaning and decontamination.

*VII-C-3-c. Penetrations into the production area shall be sealed to permit space decontamination.

*VII-C-3-d. All utility services entering the production area shall be protected against contamination.

*VII-C-3-e. Handwashing facilities equipped with foot-, elbow-, or automatically operated valves shall be located at each major work space and near each primary exit.

*VII-C-3-f. A shower facility shall be provided. This facility shall be located in close proximity to the production area.

*VII-C-3-g. The capability for sterilization of contaminated wastes shall be provided within the production area.

VII-C-3-h. Equipment used for the propagation, inactivation, or other processing of volumes greater than 10 liters of organisms containing recombinant DNA molecules shall be isolated by a curb or similar architectural feature of such size as to contain the total liquid volume contained in the equipment.

*Not required for P2-LS.

VII-C-3-i. Floor drains serving a curbed or similar area shall be sealed during periods when production is in progress.

*VII-C-3-j. The production area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the production area shall not be recirculated to other areas of the building. The exhaust air from the production area may be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

*VII-C-3-k. The exhaust air from biological safety cabinets and other containment units, as well as exhaust gases from production equipment shall be treated by a passage through a HEPA filter prior to discharge to the outdoors. Each air treatment device shall be served by an individual exhaust blower and shall be operated so as to avoid any interference with the air balance of the production area.

*Not required for P2-LS.

SUPPLEMENT TO PART VII

A supplement to Part VII of the NIH Guidelines will be prepared to provide recommended protocols for validating decontamination procedures and for certifying the integrity of containment features of equipment used in large-scale production or processing of organisms containing recombinant DNA molecules.

The NIH would welcome suggestions concerning protocols or other topics that would be of value for inclusion in the proposed Supplement. Suggestions should be sent to ORDA.

LILLY RESEARCH LABORATORIES
DIVISION OF ELI LILLY AND COMPANY
INDIANAPOLIS, INDIANA 46206

I. S. JOHNSON, Ph.D
VICE PRESIDENT

November 30, 1979

Dr. Donald S. Fredrickson
Director, National Institutes of Health
Room 124, Building 1
National Institutes of Health
Bethesda, MD 20014

Dear Dr. Fredrickson:

RE: PROPOSED PART VII - PHYSICAL CONTAINMENT GUIDELINES FOR LARGE-SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES AS NOTED IN 44 FED. REG. 63074 (NOV. 1, 1979)

We submit herewith the views of Eli Lilly and Company. Lilly is engaged in the research, development, and production of pharmaceutical products for human and animal health. The current research programs at Lilly include the utilization of recombinant DNA technology. Lilly was the first industrial firm to register, submit, and receive approval for a large-scale experiment under existing NIH Guidelines. Consequently, the company has an interest in this proposal.

We have examined the proposed physical Guidelines for large scale uses of organisms containing recombinant DNA molecules and are pleased to have the opportunity to comment upon them.

We believe the Working Group has made a sincere and thoughtful attempt to develop such physical Guidelines but feel that (1) they reflect extension of small-scale laboratory Guidelines, (2) they do not adequately reflect appropriate experience, knowledge of large-scale fermentation procedures and recognition of reliability of the equipment used in commercial production, and (3) these guidelines are ultimately tied to the scale-up approval procedures discussed at the September 6-7, RAC meeting and should be reviewed in light of consideration and comments relative to those procedures.

Only two physical containment levels are recognized by the Working Group, i.e., P2-LS and P3-LS. This approach continues to perpetuate the misconception of increased volume being equivalent to increased risk. It has been documented that the 10L level was arbitrarily selected in both the U.K. and U.S.

because of laboratory scale convenience and not risk. The RAC recognizes experiments at the P1 level and is considering host vector systems at the HV0, EK0 level. At the September 6-7, 1979, meeting the RAC recommended exclusion of all E. coli K12 experiments (excluding over 10L) from the Guidelines. In view of these considerations and in view of the physical reliability and stability of large-scale fermentation equipment it is inconsistent to recognize only two levels of physical containment (P2-LS and P3-LS). This clearly fails to recognize the containment of the vessel itself which may be superior to the containment features added by the laboratory environment of either of those categories. We believe there should be at least three containment levels, i.e., P1-LS, P2-LS, and P3-LS.

Since a number of criteria put forth by the Working Group on physical containment Guidelines clearly reflect some lack of experience, knowledge and perception of operation and reliability of modern large-scale fermentation equipment, we strongly urge and recommend the appointment of an industrial advisory group reflecting the appropriate experience. A model and precedent for such a group can be found in the GMAG organization in the U.K. The availability of such a group as a technical advisory resource should be available to or represented on the RAC as we move towards large-scale experiment, development, and production utilizing recombinant organisms.

In addition, we would make the following specific comments on the draft:

p. 3 -- VII-B-1-g -- We feel laboratory coats should meet this requirement at the P2-LS level. Operators have to leave the area for meals, visit restrooms, etc.

VII-B-1-l -- Validation of procedures should be adequate for demonstration of the inactivation of the organisms. Delete that part of the last sentence beginning with the word "inactivation" and replace it with "validated procedures for inactivation of the organisms have been employed".

p. 4 -- VII-B-2-a -- HEPA filters need not be designated. We recommend insertion of "filters capable of sterilizing or equivalent procedure". In some cases incineration should be considered. HEPA filters are fragile, will not hold up to steam sterilization, etc. The RAC has already approved units with equivalent filters.

p. 5 -- VII-B-2-b-(2) -- Use of the term "observation" is inappropriate. A statement recognizing the validity of "sensing or monitoring device or system" should be inserted. In addition, there should be recognition of pre- and post- use validation [as for example, on line dioctyl phthalate (DOP) testing] rather than observation or continuous monitoring "during" operation.

VII-B-2-b-(4) -- The use of the term "disassembly" fails to recognize the construction of large-scale fermentation vessels. Standard production facilities include the CIP concept for cleaning in-place without disassembling. "Disassembly" should be deleted.

p. 6 -- VII-B-3-a -- The phrase "in the same building... molecules" should be deleted. It is adequate that an autoclave is available for batch treatment. The transfer of materials is frequently an on-line operation utilizing piping and could involve transfer between buildings.

VII-B-3-b -- The requirement for a "curb or similar architectural feature" again clearly fails to recognize the construction and capability of large-scale fermentation vessels. In our experience of over 35 years of use of equipment of this type, such a vessel has never ruptured. These stainless steel vessels provide rapid in-place chemical sterilization. This procedure plus pumping contents into a harvest vessel for inactivation is far superior in terms of safety to the concept of allowing the contents to flow into a curbed area followed by chemical inactivation.

VII-C-1-b -- This section should be modified to read "Persons entering the production area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover and shoes or shoe covers".

p. 8 -- VII-C-1-k -- The phrase "except as necessary for access" should be inserted following the word "closed".

VII-C-1-o -- The concept of validation of procedures should be adequate for demonstration of the inactivation of the organisms as in our suggestion for VII-B-1-1.

p. 9 -- VII-C-2-a -- As in our suggestion for VII-B-2-b, HEPA filters should not be designated. "Filters capable of sterilization" should be inserted and incineration can be considered as an alternative.

VII-C-2-b -- Insert the word "viable" immediately ahead of organisms in the first sentence.

p. 10 -- VII-C-2-b-(2) -- As we suggested for VII-B-2-b-(2), "sensing or monitoring system or device "should be substituted for the term "observation".

VII-C-2-b-(4) -- "disassembly" should be deleted as recommended for VII-B-2-b-(4).

VII-C-2-b-(6) -- The equipment design may include processing the recombinant organism through killing; beyond this point the organisms would not be viable and design considerations would be exempt from the Guidelines.

p. 11 -- VII-C-3-b -- The surfaces required are not necessarily consistent with a production facility. The design of equipment for complete containment should eliminate the necessity for such a feature.

VII-C-3-c -- This type of sealed facility is only required for P4 laboratories. It is inconsistent to require it for P3-LS.

VII-C-3-g -- The phrase "shall be provided within" should be deleted and "shall be available to" substituted.

VII-C-3-h -- Our comments on "curbs" in section VII-B-3-b are applicable here as well. The requirement for "curb or similar architectural feature" again clearly fails to recognize the construction and capability of large-scale fermentation vessels. In our experience of over 35 years of use of equipment of this type, such a vessel has never ruptured. These stainless steel vessels provide for in-place sterilization. This procedure plus pumping contents into a harvest vessel for inactivation is far superior in terms of safety to the concept of allowing a vessel's contents to flow into a curbed area followed by chemical inactivation.

p. 12 -- VII-C-3-k -- Following the word "filter", the phrase "or its equivalent, i.e., incineration, etc." should be inserted.

Finally, we would like to reiterate some of our concerns over the scale-up approval procedures as represented by the draft of said procedures dated July 19, 1979, and the discussion during the RAC meeting of September 6-7, 1979.

Our statements are stimulated by an impression that the proposed procedures do not clearly recognize the need for rapid large-scale development not to mention production. We also feel that there must be a point after appropriate experience with pilot plant and production scale fermentation of K12 organisms when these procedures can no longer be considered an experiment.

In terms of the responsibility of the Director we would like to propose that the RAC in accordance with IV-E-1-b-(3)-(d) advise the Director that experiments which have a clear precedent of prior RAC approval, could be approved by the local IBC. Notification of this decision could be made by the local IBC to ORDA, the Director and/or a Working Group. In addition, we would propose that new experiments at the P2 HV1 level or below, not involving a request for reduction of containment level, would only require local IBC approval. Again, ORDA or a Working Group could receive notification of this approval. Thus, at these levels and below, a request would not be filed with ORDA, but ORDA would receive notification. This notification assumes clear acknowledgment and approval of the applicant's laboratory procedures, experience, etc., mentioned in 1 e. and f. of the proposed procedures.

We also believe that once an experiment for more than 10L has been approved by the RAC or by the procedures we have just outlined, that further expansion in volume be approved by the local IBC with notification to ORDA or a Working Group. We feel strongly that if an experiment is safe, risk is not proportional to the volume because of the safety and reliability of the industrial equipment normally used at these levels; but if risk exists at all, it is more apt to be proportional to the number of times the experiment is done. We would like to emphasize that these experiments are not done in Erlenmeyer flasks which can be dropped, broken, etc., but in rigid stainless steel tanks with a number of safety features.

We would make the following comments on the four agenda items which in some cases are extensions of those already made.

1. The 10L limit should refer to the volume held in a single container. If that container reliably contains the 10L experiment, any additional containers or comparable construction surely will contain that same volume equally well.

2. We do not feel an intervening category between 10L and 100L need be created. We feel strongly that the reliability of the design and the experience with that equipment is the overriding pertinent fact. As indicated in our preceding comments, we do not perceive 10L experiments to be less safe at higher volumes.

3. We have already indicated that we feel IBC's should be able to approve with concurrent advisory notice to the Director of ORDA, any large-scale experiment for which RAC approval has been previously obtained as well as new experiments at the P2 HV1 level. These proposals do assume documentation of the appropriate procedures, facilities, experience, etc., of the laboratory where these experiments will be carried out.

   We would like to propose that RAC subcommittees or Working Groups have the responsibility to approve or recommend approval to the Director, unless within 15 days after receipt of a new submission by the whole RAC, there is a clear preponderance of concern. Approval could then be handled by telephone by the subcommittee or Working Group.

4. Fortunately, the RAC as currently constituted represents several scientific disciplines as well as the public interest. These disciplines could be appropriately supplemented with additional representation, particularly in considering submissions which clearly are breaking new ground as opposed to replowing old. In the former case we feel representation from the fields of infectious disease, *i.e.*, microbial pathological processes, epidemiology, chemical-biochemical engineering, and fermentation technology particularly should be represented on the Working Group or subcommittee. We believe in many or most instances these

areas could be represented by as few as three individuals. They should be drawn from whatever institutions the expertise might lie. Since industrial firms with the appropriate experience will be the largest potential users and practioners of the art, it is possible that industry would represent at least one such source. We would hope it would not necessarily be excluded. As a possible alternative, albeit less desirable, some of the representatives could function in an advisory or consulting role to a subcommittee or Working Group.

Sincerely,

Irving S. Johnson

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
ROCKVILLE, MARYLAND 20857

January 17, 1980

Dr. Elizabeth Milewski
Office of Recombinant DNA Activities
Bldg. 31, Room 4A52
Bethesda, Maryland 20205

Dear Dr. Milewski:

Enclosed are the Food and Drug Administration's comments on proposed Section VII of the Guidelines entitled "Physical Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules." The comments were prepared by Dr. Armand Casola, who is a member of our Bureau of Drugs. We feel that incorporation of his recommendations (particularly his item #7) into the Guidelines will contribute significantly to the integrity of large-scale production systems.

We would also like to request that we be kept informed of your response to Senator Stevenson's letter of November 21 with regard to the proposed legislation which would legally require the registration of all recombinant DNA work (research) which is not presently subject to the Guidelines.

Sincerely yours,

Tim Henry

Timothy J. Henry, Ph.D.
Staff Science Advisor (HFY-31)
Office of Health Affairs

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Dr. Tim Henry, HFY-31
Office of Health Affairs

DATE: January 14, 1980

FROM : Supervisory Chemist
HFD-140

SUBJECT: Comments: Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules

In answer to your questions as asked on the Transmittal Slip, it appears that the Guidelines are compatible with large scale pharmaceutical fermentation procedures. As to completeness, the comments which follow will address this query. The Guidelines are not naive but appear to be seriously considered and well organized.

I recommend the following additions and modifications for your consideration:

1. All entrances to the production area should be equipped with flashing red warning lights which direct attention to the universal biohazard sign. This applies to P2-LS Level, page 2 item VII-B-1-b and P3-LS Level, page 7 item VII-C-1-e.

2. A statement regarding the use of face masks to protect against inhalation of viable organisms is needed.

3. Additional precautions may be needed under VII-B-1-n and VII-C-1-q in the transportation of the durable leak-proof container. Although it is sealed, should it not be decontaminated on the outer surfaces prior to transportation from the production area?

4. Under VII-B-3-c and VII-C-3-i the floor drain system should be equipped with a removable, leak-proof holding tank in-line as an added precaution against contamination of the environment.

5. Under VII-B-2-a and VII-C-2-a the HEPA filter should be autoclave-sterilized or incinerated if to be discarded. If not discarded, the filter should be autoclave-sterilized before re-use.

6. There should be a slightly negative air pressure in the operations area so that air from outside is drawn in and air inside the operations area does not escape when doors or other ports are opened.

7. The exhaust air from the production area (VII-C-3-j) should not be discharged directly to the outdoors without filtration and treatment. The air should be filtered through a HEPA filter and the ducts on the effluent side of the filter should contain Ultra Violet disinfection systems to treat the filtered air as an added safety precaution. Electronic filters or precipitators should be considered also.

Armand R. Casola

Armand R. Casola, Ph.D.

cc: HFD-140
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Dr. Elizabeth Milewski
Dept. of. H.E.W.
NATL. INST. HLTH.
Bethesda Md. 20205

1/20/80

Dear Dr. Milewski,

Your letter of the 9th, delivered on the 15th requesting comments on part VII required a rather rapid answer. Thus, the informal response.

In reviewing the document I found that there were several sections that required more extensive discussion of the program before I could offer adequate comments. Nonethe less I have assumed you wish to stay on schedule and have sent along comments even if incomplete.

I will assume that further opportunity for comment will exist in the public distribution period. I would appreciate being on your mailing list for this distribution.

Should there be further discussion required I will be in my office in the latter part of next week.

Cordially
Mark Chatigny

Encl. Comments

MARK A. CHATIGNY
15926 Via Toledo
San Lorenzo, California 94580
(415) 276-4307

20 JAN 1980

Comments on 11/14/79 Draft. Part VII – Guidelines for large scale uses of organisms containing RDNA.
Word – underline is addition, ~~word~~ – line out is delete
TP

VII-B-1-b --- access doors to the research and production
-1-e -- An effective insect and ---
-1-h -- is in progress. Service doors leading directly to out doors shall remain closed and locked.
-1-j --- aerosol formation. Rotating seals or other high speed machinery shall have special designs or provision for aerosol containment. (See VII-B-2-a)

1-h -- in ~~a~~ durable leak-proof container(s) which are ~~is~~ sealed.

VII-B (New) (Proposed Additions)

(1) Closed vessels for propagation and growth of viable organisms containing RDNA molecules shall be operated at atmospheric or slightly negative pressure. This pressure shall be maintained during temperature buildup on a sterilizing cycle.

(2) Exhaust from enclosed vessels shall be filtered through moisture resistant HEPA or equivalent filters that allow penetration of not more than $1/10^7$ of 0.3-1.0 μm particles.

(3) Sufficient disinfectant shall be available to inactivate all viable organisms present that

(3) Cont.

- Contain recombinant RDNA.

(4) Inactivation and sterilization procedures ~~that~~ used shall be demonstrated to be effective using non-debilitated strains of the host organisms in the specific equipment to be employed.

(5) Sample collection or process additives during propagation and growth of organisms containing RDNA molecules shall be done without violating the containment used and with control of any aerosols released.

VII-B-2

- b(2) ---observation and monitoring during ----
  - (3) --- provides for verified sterilization of all---.
  - (6)--Be individually tested --- Test organism. Records of tests and results shall be maintained on file.
  - (7) -- be individually and permanently---

Proposed New Additions

Be fabricated and operated so as to eliminate blind spaces, pockets or other voids that are not fully sterilizable.

VII-B-3.

-b ---to contain not less than the total

VII-C P3-LS level.

C-1

C-1-e --- sign including a statement of agents in use and personnel authorized to enter,

C-1-i An effective insect and ---

-j -- shall wear suitably durable impermeable protective gloves

K --- in progress. Service doors leading directly out doors shall be sealed and locked while work is in progress.

m --- of the environment. Rotating seals and machinery shall be espeially designed to prevent leakage or shall be fully enclosed in ventilated hoosings exhausted through HEPA filters.

VII-C-V Proposed New Additions

(1) Closed vessels holding viable organisms containing RDNA molecules shall be operated at atmospheric or slightly negative pressure. This pressure shall be maintained at all times including temperature buildup during sterilizing cycles.

(2) Gases exhausted from enclosed vessels shall be filtered through a moisture resistant HEPA filter or equivalent and an incineration process with a viable spore. (B. Subtilus. Vniger) reduction of not less than 10 decades assum-

(2) Cont.

-ing first order kinetics.

(3) Sterilization procedures shall be demonstrated to probability of not more than $1/10^3$ that any viable spores (B. Stearothermophilus) would be present at the end of the cycle provided the initial concentration is that expected for the host organism. First order kinetics may be used for extrapolations.

(4) Sampling and collection during propagation and growth of organisms containing RDNA molecules shall be done without violation of the containment. (also include additives). Provision shall be made to prevent release of any organisms except in full coverage safety enclosures.

(5) Inactivation procedures shall be demonstrated to be fully effective with probability less than $1/10^3$ that any viable organisms will remain. Test shall be made with non-debilitated organisms of the host strain in the specific vessels to be employed.

VII-C-2.

" (a) -- containment units that are Similarly Ventilated -----

" b-(1) -- contains viable organisms and during any decontamination or sterilization process.

" b(2) -- observation and monitoring remotely or visually during - - -

" b(3) --- provides for verified sterilization of all ----
--- environment. Particular attention shall be paid to voids, pockets, traps and unvented head spaces.

" b-(4) --- of the unit and non contaminated conditions for maintenance or repair.

(b) (5) --- utilities and service piping and wiring protected

b (6) -- Be individually tested ---

VII-C-3 Special Facility Design.

" c --- to permit liquid or vapor phase space decon
" -d --utilities and service or process piping or wiring entering...
" -h -- to contain not less than the total ---

Proposed New Addition.

(i) In any space wherein multiple growth units and accessory vessels are installed and used provision shall be made to treat each set of equipment used for a host organism strain separately with full facility isolation regarding access, ventilation, and containment. Alternatively, the use of any system ~~at~~ highest containment level in a space shall require all other equipment to be used at that level. (5)

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Coalition for Responsible Genetic Research

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Executive Director

January 21, 1980

The Honorable Patricia Roberts Harris
Secretary
Department of Health, Education and Welfare
Washington, D.C. 20201

Dear Secretary Harris:

Responding to the request for comment on large-scale recombinant DNA projects, we wish to register the following points:

1. The upper limit of 10 liters of culture of recombinant DNA organisms has prevailed in NIH Recombinant DNA Guidelines since June 23, 1976. Great pressure for exemptions from this limit is being brought to bear by those whose interest lies mainly in the industrial application of this controversial technology.
2. Recent NIH risk assessment experimentation indicates greater hazard than previously thought:
    a. recombinant DNA molecules survive for four days in sewage and the human gut[1]
    b. naked polyoma DNA can cause infection in sterile mice[2]
    c. gene-splice products can cause tumors in experimental animals[3]
3. Risk assessment of large-scale projects has not been pursued although industrial scale-up would involve volume-oriented production of recombinants carrying hormones (insulin, somatostatin, etc.) and other human substances (e.g. interferon). The impact on human and other populations is not known (i.e. anaphylaxis, auto-immune diseases, etc.) and investigation has yet to be initiated.
4. Expanding the volume of production sharply increases the chance for biologic mishap.

Before exemptions from the 10 liter limit can be considered, experimentation to assess the risks of large-scale production must be carried out. Such investigation needs, at minimum, to include the following:

---

[1] S. Levy and B. Marshall, "Survival of E. coli Host-Vector Systems in the Human Intestinal Tract", Recombinant DNA Bulletin 2/7/79

[2] B. Rosenberg and L. Simon, "Recombinant DNA:Have Recent Experiments Assessed All the Risks?", NATURE, 282, December 20-7, 1979.

[3] "Molecular Cloning of Polyoma Virus DNA in Escherichia coli; Oncogenicity Testing in Hamsters", SCIENCE, 205, March 2, 1979.

1. assessment of the disposal of large quantities of recombinant organisms into effluent systems
2. pharmacologically-active proteins produced by gene-splicing in large quantities should be adequately pre-tested for effects on humans and the environment.
3. experimentation to include anaerobic bacteria and wild strains of E. coli.

If the data derived from these investigations indicate that, with specified safeguards, it would not be premature to proceed, then the following should be included among those requirements for projects exceeding the 10 liter limit:

1. sufficient evidence to demonstrate that the subjects for cloning would not be harmful
2. prohibition of release of recombinant organisms into the environmant (drains, effluent, waste, etc.) without meticulous testing to ensure that all organisms are inactivated
3. ongoing health surveillance and maintenance of health records of employees

It seems to us patently clear that protection of the public health requires that industry comply with NIH regulations. Historically, industrial self-policing has never worked. It is not likely that there is any reason to suspect a change in this area, nor can we permit ourselves the luxury of experimentation with voluntary compliance; the technology of recombinant DNA does not allow much margin for error. We wish, therefore, to underline our unequivocal support for required compliance with regulation by industry, which was voted for by the NIH Recombinant DNA Advisory Committee.[5]

We need to keep in mind the tragic failures of the nuclear energy industry: only by open, full disclosure of facts and data, regulation of public and private sectors, and a cautious approach will we be able to avoid a biological Three Mile Island.

Reducing laboratory containment standards and permitting exemptions without supporting data constitute neither a cautious nor a scientific approach. We hope that your tenure in office will be marked by a change from current NIH philosophy and practice to one of caution that would be more appropriate to an agency whose "mission is to improve the health of the American people".[4]

Yours very truly,

Francine Robinson

Francine Robinson
Executive Director

FRS:fh

---

4. U. S. Government
   Organizational Manual

5. S. Krimsky and D. Ozonoff,"Recombinant DNA:Scope and Limits of Regulation", American Journal of Public Health, December 1979.

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

TO : Dr. William J. Gartland
Office of Recombinant DNA Activities
National Institutes of Health

DATE: January 25, 1980

THRU: Melvin Myers, Director OPPE

FROM : Health Scientist, OPPE/PAB

SUBJECT: NIOSH comments on the NIH Draft Part VII, Physical Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules.

We have examined, the Draft Part VII, Physical Containment Guidelines for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules and are pleased to comment upon them. Our comments will be confined at the present time to four areas of concern.

1. Research Guidelines vs Guidelines for Commercial Applications
2. Assessment of Increased Risk
3. Format and language
4. Occupational Safety and Health

Research Guidelines vs Guidelines for Commercial applications

The draft guidelines for large-scale uses appear to be developed from the perspective of merely expanding the scale of a laboratory research operation. They do not address the different circumstances, procedures, and equipment found in commercial operations. The draft of Part VII does not reflect an appreciation of the fact that laboratory technicians and operators are WORKERS; they do not address the fact that the laboratory or production site is an occupational work setting.

In a letter to Dr. Emmett Barkley from Dr. Robert McKinney (RAC consultant) dated July 26, 1979, various concerns over the safety of fermentors for the propagation of potentially hazardous microorganisms were raised. In this letter, Dr. McKinney addressed several phases of large-scale fermentor operations where increased hazard may exist such as: 1) charging or inoculating the fermentors, 2) collection of in-process samples, 3) harvesting of the raw product, and 4) processing of the product. Discussion of these concerns are absent from Part VII. Dr. McKinney felt management of potential hazards associated with fermentors could be accomplished with "existing equipment, methods, and technology", but these controls were not delineated or recommended in Part VII.

Similar attention should be focused on other aspects of large-scale production which would differ from those of laboratory research. Such concerns pertain to equipment, size, design, and the interface with other process; they also may include possible differences in employee training, education, and motivation.

The Guidelines focus attention on the control of aerosol-producing procedures (although not exclusively). They do not sufficiently address the potentials for accidents-and likely (or unlikely) consequences - that may occur in large-scale commercial operations.

Assessment of Increased Risk

There is no discussion of the assessment of increased degree of risk assumed to be associated with work greater than 10 liters. Where does the increased risk come from; what is its nature? Does the degree of risk and the nature of hazards change as the size of the experiment or operation increases? Until the nature of the hazards are discussed and assessed for different magnitudes of production, the associated risks and attendant control measures are quite open to question.

Format and Language

The format and language of the draft guidelines for physical containment of large scale uses are similar to, but not entirely consistent with, the NIH Guidelines. As discussed in comment (1), it is obvious that the guidelines for large scale uses are derived from laboratory research operations, therefore, at the very least, the format and language for Part VII should be carefully reviewed and edited to be consistent with that of existing Guidelines. A great deal of explicitness in procedures and requirements now incorporated in existing Guidelines has not been incorporated in the draft guidelines for large scale uses. For example, in the draft Part VII, the type of protective clothing, and equipment or manipulations that produce aerosole are not specified to the same degree or in the same terminology as the existing Guidelines. Until consistency is achieved, it does not seem worthwhile to comment further on specific provisions.

Occupational Health and Safety

Personnel and environmental surveillance and routine monitoring, as well as occupational safety and health considerations are essentially absent from the draft for Part VII. Routine monitoring provides essential information on the efficiency of control technology and, with rigorous record keeping, can contribute invaluable data toward risk assessment and control. The following factors are pre-requisite elements of such a program.

a. Registry of potentially exposed individuals

b. Work area monitoring program, including air and surfaces, for viable recombinant DNA host-vector organisms.

c. Direct personnel monitoring for host-vector organisms, and if colonization has occured, further testing for the expression of the relevant gene product may be appropriate.

d. Occupational and medical histories which should include a determination of existing factors which might place certain workers at increased risk.

The NIH Laboratory Safety Monograph discusses various possible components of a health surveillance and work practices program. This monograph is not mentioned in Part VII. It most certainly should form a basis for development of work in this area and be referenced where appropriate in Part VII.

Since work with recombinant DNA molecules is relatively recent, and as the host-vector organisms and genome of interest differ between protocols, it should be recognized that effective monitoring can present unique problems and research in this area is essential and urgently needed.

Although the introductory paragraph to the draft of Part VII states "all provisions of the NIH Guidelines shall apply to large-scale research and production activities", critical areas such those commented on above should be addressed in Part VII.

Seth Pauker

Seth Pauker

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN 49001, U.S.A.
TELEPHONE (616) 323-4000

January 25, 1980

Dr. D. S. Fredrickson
Director, National Institutes of Health
Room 124, Building 1
National Institutes of Health
Bethesda, MD 20014

Dear Dr. Fredrickson:

RE: PROPOSED PART VII - PHYSICAL CONTAINMENT GUIDELINES FOR LARGE-SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES AS NOTED IN 44 FED. REG. 63074 (NOV. 1, 1979)

We submit herewith the views of The Upjohn Company on the proposed physical containment guidelines for large scale uses of organisms containing Recombinant DNA molecules.

We believe the Large Scale Working Group has made a sincere attempt to develop physical guidelines for large scale cultures but feel that the proposed guidelines do not adequately reflect the reality of present day large scale production processes.

The working group has only recognized two physical containment levels i.e. P2-LS and P3-LS. The proposals for P2-LS containment are in a large measure desirable and applicable for the scale-up or production of Recombinant DNA organism and products which pose a perceived biohazard. There are, however, recombinant organisms and products which do not present any perceivable biohazard, for example recombinant organisms produced through self-cloning or through cloning DNA from closely related saprophytic organism i.e. a streptomycete containing DNA on an indigenous plasmid from another saprophytic streptomycete. Thus, the proposed guidelines should provide a category, e.g. P1-LS, which would cover recombinant organism with no perceived hazard. One could visualize that the RAC could derate organisms to P1-LS by procedures similar to those used currently to derate the physical and biological containment level of recombinant organims. We would further propose that P1-LS be equated to the containment practices of current standard large scale fermentations. These would essentially be equivalent to the requirements given in the guidelines for P1 physical containment levels (II-B-1-a through IIB-1-C).

In our view the P3-LS provisions are probably unnecessary. Any process being scaled up in large fermenters or being put into actual production should be engineered into safe hosts and sufficiently characterized to allow derating to P2-LS or P1-LS containment conditions.

A number of the proposed laboratory practices advocated by the Working Group on large scale physical containment guidelines clearly illustrate the lack of experience , perception and knowledge of large scale fermentations. We feel that a meaningful understanding of the operation, design and reliability of modern large scale equipment is necessary and strongly recommend that an industrial advisory group with the appropriate experience be appointed to assist in this task.

In addition, we would specifically recommend the following changes in the proposed laboratory practices for P2-LS.

p. 3 -- VII-B-1-g. We feel appropriate laboratory coats should meet containment needs as long as they are not worn out of the production facility.

VII-B-1-1. We feel that material from fermenters should be transferable to other facilities if validated procedures are followed for inactivation of organisms.

VII-B-1-m. We are unclear as to the meaning of the phrase "...prior to processing for reuse." If a fermenter is to be reused immediately is it necessary to sterilize the tank empty before a fresh fermentation, medium is added and sterilized?

p. 4 -- VII-B-2-a. We recommend inserting "viable" in the phrase "...procedures involving viable organisms containing recombinant DNA molecules." Also, HEPA filters should not be specified, as other filters with more desirable properties are available which accomplish the desired objective.

p. 5 -- VII-B-2-b-(2). We feel that the "integrity of containment" can be recognized and monitored by a number of sensing and monitoring devices and that use of the term "observation" may be too narrow and restrictive.

VII-B-2-(4). To talk about disassembly of large scale fermentation equipment does not convey an understanding of the design, construction and operation of such equipment. We recommend deletion of "disassembly," i.e. --- facilitates cleaning and maintenance to assure integrity of ---.

p. 6 -- VII-B-3-b. The requirement for a "curb or similar architectural feature" does not recognize the construction and features of large scale fermenters. Based on our many years of experience, we feel that the possible rupture of stainless steel fermentation vessels is so remote as to warrant its exclusion from consideration. We do not feel that the P3-LS level of containment is a realistic entity as discussed before. We feel a much safer approach is to require sufficient characterization studies of the recombinant DNA organism to allow its derating to P2-LS or P1-LS.

Sincerely,

James D. Punch
John H. Coats

James D. Punch, Ph.D.
John H. Coats, Ph.D.
Co-chairman, Upjohn Recombinant
DNA Advisory Committee

/dsc

GMAG

Genetic Manipulation Advisory Group
at Medical Research Council
20 Park Crescent, London W1N 4AL

telephone 01-636 5422

reference S604/26

29 January 1980

Dear Bill,

Thank you very much for your letter of the 20 December and the draft NIH guidelines relating to large-scale use of genetically manipulated organisms.

The present position in the UK is that GMAG has only given preliminary advice on one 'large-scale' proposal. The Group have received a preliminary proposal for 'large-scale' work from a Pharmaceutical Company and the Group has had exploratory discussions with them. However, GMAG as you know has decided to consider 'large-scale' work under a different procedure compared to the NIH. Until the plant is complete the Group will not give their formal advice. At present the Company has not constructed the plant although it is fair to say that they have provided information on the biological systems, plant design and safety procedures etc. that they intend to use. GMAG has taken the view that it will not categorise 'large-scale' work along the physical containment requirements which have been recommended for laboratories. It will review each 'large-scale' application on a case by case basis and on the information provided regarding the physical, biological, safety and operating procedures it will advise on whether the Group consider the work can be safely carried out. The Group has, therefore, not made any statement to date relating to the physical containment for such work.

With regard to the draft guidelines I am sure that GMAG would expect that most of the recommendations in your draft guidelines are incorporated into the operating procedures for work carried out in the UK with perhaps one or two exceptions. For example:

i) V11-B-2-a - With the exception of the laboratory aspects of the work GMAG would not necessarily insist on a HEPA filtered system. . If evidence was provided that some other form of sterilisation (such as steam or ignition of gases) was effective then the alternative would be accepted. This also applies to V11-C-3-k.

ii) V11-C-3-j - In an industrial environment such requirements may well prove very difficult to implement in practice. The necessity to control and the extent of any control of the ventilation system must very much depend on the particular organism etc. For this reason the GMAG has once again decided not to codify the physical containment necessary for large-scale work but to adopt a much more flexible approach.

The GMAG also intend to place emphasis on a site visit to assess the overall safety of the operation.

...../

I enclose a copy of GMAG Note 12 for your information which explains the Group's attitude towards large-scale work.

I hope the above comments are of some value. Please let me know if you would like any further information.

Yours sincerely

Keith

K Gibson

Dr William Gartland
Director, Office of Recombinant DNA Activities
National Institute of Allergy and Infectious Diseases
Bethesda
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GMAG NOTE 12

LARGE SCALE USE OF THE PRODUCTS OF GENETIC MANIPULATION - WORK INVOLVING VOLUMES OF 10 LITRES OR MORE

---

1. GMAG through its Note 8 (29 March 1979) and Note 7 (Revised May 1979) announced that it would wish to give special consideration to proposals to do scale-up work involving the growth of self-propagating products of genetic manipulation in volumes of 10 litres or more.

GMAG has decided that it would wish to consider all proposals involving 'scale-up' in conjunction with a site visit. As a consequence all work involving 'scale-up' will be considered on a case by case basis. Initial proposals should be submitted on either the proposal forms A and B detailed in the Genetic Manipulation Regulations (S1 1978 No.752), or in the form of a detailed letter. The precise form of the submission should be discussed with the GMAG Secretariat before a formal request for advice is made.

Guidance Notes

2. It is important in the assessment of proposals to do scale-up work to use the term 'scale-up' with strict regard for its meaning in this context. The expression is usually applied in the development of chemical, pharmaceutical and biological production processes in which the scale of the operation, the same operation, is translated from that of the bench to the pilot plant and then to the full-scale industrial plant. In the context of the GMAG responsibility, scale-up will involve the very significant change from the manipulation of the genetic material to the use of the resulting host vector system.

3. A consequence of the change from manipulation to use is that any risks involved will not necessarily remain the same and a different type of assessment will be required for these changed circumstances.

4. It has proved possible and convenient to assess by various means the conjectural hazards of genetic manipulation and to assign the proposed experiment to specified conditions of containment in identified categories from I to IV.

5. The different conditions of the industrial scale make it less appropriate to attempt to categorise proposals according to defined levels of containment. A preferable approach is to integrate the biological characteristics of the system with the physical characteristics of the plant, to review arrangements for the running of the plant for example by reference to an operations manual and to make an overall assessment for acceptance or rejection of the proposal. Acceptance may, of course, be qualified provided appropriate changes are made to eliminate an unacceptable feature or to decrease any associated hazard.

6. Vaccine production and antibiotic production are two well-established industrial biological processes which provide the design of plant and the operational experience appropriate for the large-scale culture of a self-propagating product of genetic manipulation and the subsequent extraction of its active principle. There is little to distinguish these processes from those of chemical engineering in which effective design, efficient fabrication and skilled operation provide a very high degree of containment.

7. When considering proposals which involve 'scale-up' the GMAG will wish to have information additional to that usually required on the usual proposal forms. In addition to such details as the nature of the biological host/vector system and the inserted DNA sequences the GMAG will ask for information on the extraction of the product, details of the building and fermentation plant (such as the structure and operating procedures), the disposal of waste, emergency procedures, the training of operatives and safety procedures (including details of the local safety committee, biological safety officer and medical supervision).

8. Proposal for large scale work that have implications for commercial property or patent protection may be submitted under the Group's special confidentiality scheme. Further details of this scheme are obtainable from the Group's secretariat.

9. The GMAG Secretariat at the MRC Headquarters, 20 Park Crescent, London W1N 4AL, will give advice to any person wishing to submit a proposal involving scale-up to the Group.

November 1979

GMAG

Genetic Manipulation Advisory Group
at Medical Research Council
20 Park Crescent, London W1N 4AL

telephone 01-636 5422

reference

31 January 1980

Dear Dr Gartland,

Guidelines for large-scale use

I apologise for taking so long to respond to the invitation contained in your letter of the 20th December to Dr Keith Gibson.

When I accepted the invitation to succeed Sir Gordon Wolstenholme as Chairman of GMAG, I anticipated that it would soon be necessary to move into the area of "scale-up". I, therefore, reconstituted the GMAG Sub-committee for this subject and decided to chair it myself. It appeared to me to be of fundamental importance to ensure that our biotechnology industry was permitted to operate with the minimum of constraints while maintaining an adequate degree of safety; also, that if the benefits of genetic manipulation were to be fully realised, we must be prepared to move rapidly from the bench to the industrial scale.

As a result of our discussions at Sub-committee level followed by those at GMAG, we were in unanimous agreement on certain principles which are reflected in our GMAG Note 12 (copy enclosed).

These principles are:

1. "Scale-up" in the context of genetic manipulation is different from the accepted use of the term in, say, the chemical industry. It will invariably mean use not manipulation.
2. Any risks related to use will not necessarily be the same as those related to manipulation.
3. A different type of assessment may well be required because of the different risks.
4. The skill and experience of chemical engineers is well proven and the type of large-scale plant used for antibiotic production provides a pattern for that required for the industrial application of the results of recombinant DNA research.
5. The very high cost of mounting the commercial operation of large-scale biotechnology, eg. the cost of the medium to fill an industrial-scale fermenter and the consequences of any contamination tend to ensure that the safety desirable for the growth of genetically manipulated organisms is likely to be achieved.
6. For these reasons, GMAG decided that the use of categories of containment was quite inappropriate for large-scale use.

7. The accepted approach is outlined in paragraph 5 of GMAG Note 12, namely, that an individual assessment must be made in each case, taking into account the biological characteristics of the system and the physical characteristics of the plant combined with an appraisal of the competence of the management and the training and skill of the operators. This appraisal involves making a site visit. We regard this as being essential whereas I understand that this may present some difficulties for you.

I note that in the first line of Section VII of your guidelines where it refers to "..... large-scale uses ....." your cite "the laboratory practices". This may account for some misunderstanding between us. We believe that the optimum move in scale is to go as directly as possible to the final industrial volume. We would be happy to go with our GMAG Note 12 and our check list to inspect any plant with fermenters of some thousands of litres capacity.

I feel very strongly that our approach is correct and that it is a mistake to include use with manipulation as you and the EEC propose in your respective drafts.

In your letter to Dr Gibson, you refer to the comments made by Dr Irving Johnson about levels of containment lower than "B2-LS" being approved in the UK for large scale work. You will appreciate that this comment is irrelevant but I share his misgivings about the "architectural curb" (VII - B -3 - b and VII - C - 3 - h). This requirement is unrealistic except for a scale of a few hundreds of litres whereas we must be planning for a scale of some thousands of litres.

Yours sincerely,

William Henderson

William Henderson

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GMAG NOTE 12

LARGE SCALE USE OF THE PRODUCTS OF GENETIC MANIPULATION - WORK INVOLVING VOLUMES OF 10 LITRES OR MORE

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GMAG has decided that it would wish to consider all proposals involving 'scale-up' in conjunction with a site visit. As a consequence all work involving 'scale-up' will be considered on a case by case basis. Initial proposals should be submitted on either the proposal forms A and B detailed in the Genetic Manipulation Regulations (S1 1978 No.752), or in the form of a detailed letter. The precise form of the submission should be discussed with the GMAG Secretariat before a formal request for advice is made.

Guidance Notes

2. It is important in the assessment of proposals to do scale-up work to use the term 'scale-up' with strict regard for its meaning in this context. The expression is usually applied in the development of chemical, pharmaceutical and biological production processes in which the scale of the operation, the same operation, is translated from that of the bench to the pilot plant and then to the full-scale industrial plant. In the context of the GMAG responsibility, scale-up will involve the very significant change from the manipulation of the genetic material to the use of the resulting host vector system.

3. A consequence of the change from manipulation to use is that any risks involved will not necessarily remain the same and a different type of assessment will be required for these changed circumstances.

4. It has proved possible and convenient to assess by various means the conjectural hazards of genetic manipulation and to assign the proposed experiment to specified conditions of containment in identified categories from I to IV.

5. The different conditions of the industrial scale make it less appropriate to attempt to categorise proposals according to defined levels of containment. A preferabie approach is to integrate the biological characteristics of the system with the physical characteristics of the plant, to review arrangements for the running of the plant for example by reference to an operations manual and to make an overall assessment for acceptance or rejection of the proposal. Acceptance may, of course, be qualified provided appropriate changes are made to eliminate an unacceptable feature or to decrease any associated hazard.

6. Vaccine production and antibiotic production are two well-established industrial biological processes which provide the design of plant and the operational experience appropriate for the large-scale culture of a self-propagating product of genetic manipulation and the subsequent extraction of its active principle. There is little to distinguish these processes from those of chemical engineering in which effective design, efficient fabrication and skilled operation provide a very high degree of containment.

7. When considering proposals which involve 'scale-up' the GMAG will wish to have information additional to that usually required on the usual proposal forms. In addition to such details as the nature of the biological host/vector system and the inserted DNA sequences the GMAG will ask for information on the extraction of the product, details of the building and fermentation plant (such as the structure and operating procedures), the disposal of waste, emergency procedures, the training of operatives and safety procedures (including details of the local safety committee, biological safety officer and medical supervision).

8. Proposal for large scale work that have implications for commercial property or patent protection may be submitted under the Group's special confidentiality scheme. Further details of this scheme are obtainable from the Group's secretariat.

9. The GMAG Secretariat at the MRC Headquarters, 20 Park Crescent, London W1N 4AL, will give advice to any person wishing to submit a proposal involving scale-up to the Group.

November 1979

GENETIC MANIPULATION ADVISORY GROUP

CHECK LIST for site visits to centres which are involved in the large scale production of genetically manipulated organisms

Name of laboratory or company:

GMAC reference:

Date of visit:

Visit made by:

A. BIOLOGICAL SYSTEM

		Additional Comments
Host		
Disabled	Yes ☐ No ☐	
Degree of pathogenicity if not disabled	High ☐ Medium ☐ Low ☐	
Stability	High ☐ Medium ☐ Low ☐	
pH sensitivity	High ☐ Medium ☐ Low ☐	
Sensitivity to oxygen tension	High ☐ Medium ☐ Low ☐	
Temperature sensitivity	High ☐ Medium ☐ Low ☐	

Vector/Inserted DNA

Characterisation and specificity of introduced genetic material

Mobilisation

Amplification

Subsidiary translation into protein

Culture

Batch

Continuous

Setting-up Hazard High ☐ Medium ☐ Low ☐

Antibiotic content of medium

Additional Comments

Yield - final target concentration

- final minimal acceptable concentration

Purity

Criteria for rejection

Disposal of rejected cultures

B. EXTRACTION OF PRODUCT

Inactivation of culture

Physical inactivation

Chemical inactivation

Tests of inactivation

If the culture is not inactivated

Toxicity (of culture or product) High ☐ Medium ☐ Low ☐

Pathogenicity High ☐ Medium ☐ Low ☐

Purity of product High ☐ Medium ☐ Low ☐

C. PROCESSING OF NOT INACTIVATED CULTURE

Extraction process

Tests for survival of host organism (if extraction demands its exclusion)

Bottling

Storage

Distribution

D. BUILDINGS

Type of building Satisfactory ☐ Not satisfactory ☐

Siting in relation to other accommodation Satisfactory ☐ Not satisfactory ☐

Access - restricted Satisfactory ☐ Not satisfactory ☐

- adequately notified Satisfactory ☐ Not satisfactory ☐

- security Satisfactory ☐ Not satisfactory ☐

				Additional Comments
Observation -	Window ☐	Tele-camera ☐	Nil ☐	
Size - adequate space around fermentors and other major equipment	Yes ☐		No ☐	
Floor - suitable material	Yes ☐		No ☐	
- ease of cleaning	Easy ☐		Not easy ☐	
- arrangements for cleaning	Satisfactory ☐		Not satisfactory ☐	
- arrangements for dealing with spillages	Satisfactory ☐		Not satisfactory ☐	
Essential services	Satisfactory ☐		Not satisfactory ☐	
Emergency arrangements	Satisfactory ☐		Not satisfactory ☐	
Local Code of Practice	Satisfactory ☐		Not satisfactory ☐	
Plant room pressure and atmosphere -	Ambient ☐	Positive ☐	Negative ☐	
- filtered air inlet	Yes ☐		No ☐	
- filtered air outlet	Yes ☐		No ☐	
- bacterial testing	Satisfactory ☐		Not satisfactory ☐	

E. ACCESS SUITE

Changing rooms - partial or complete changing	Yes ☐	No ☐	
- shower facility	Yes ☐	No ☐	
Partition - between clean and restricted side	Yes ☐	No ☐	
Adequate space	Yes ☐	No ☐	
Provision for storage of protective clothing	Yes ☐	No ☐	
UV light	Yes ☐	No ☐	
Door arrangement	Satisfactory ☐	Not satisfactory ☐	
Washing facilities	Satisfactory ☐	Not satisfactory ☐	

Additiona
Comments

F. WASTE DISPOSAL

By-products, effluents and wastes Satisfactory ☐ Not satisfactory ☐

Method of treatment before disposal Satisfactory ☐ Not satisfactory ☐

G. PLANT

Fermentors:

- volume Litres ☐
- material of construction

Pipe works, pumps and transfer systems:

- material of construction
- diameter

Joints - welded Yes ☐ No ☐

Pressure testing of system Yes ☐ No ☐

Effluent gas outlet:

- steralisation system Yes ☐ No ☐
- data on effectiveness of process Yes ☐ No ☐

Method of agitation or stirring:

- nature of stirrer seal Magnetic ☐ Other ☐

Fermentor operating pressure - Negative ☐ Positive ☐

Instrumentation:-

- insertion points Sealed ☐ Not sealed ☐
- permeability of sensors

Process control sampling:

- technique and frequency Satisfactory ☐ Not satisfactory ☐

Fail-safe and emergency systems Satisfactory ☐ Not satisfactory ☐

H. TRAINING OF OPERATIVES Adequate ☐ Inadequate ☐

I. GENERAL

			Additional Comments
1. .rocedure in the event of an accident:			
- record book	Yes ☐	No ☐	
- arrangements for notification	Yes ☐	No ☐	
2. Arrangements in the event of contamination of staff	Adequate ☐	Inadequate ☐	
3. Decontamination procedure:			
- spills	Satisfactory ☐	Not satisfactory ☐	
- fumigation	Satisfactory ☐	Not satisfactory ☐	
- monitoring	Satisfactory ☐	Not satisfactory ☐	
4. Separation of production cycles	Adequate ☐	Inadequate ☐	
5. Emergency/disaster procedures	Adequate ☐	Inadequate ☐	
6. Arrangements for maintenance	Adequate ☐	Inadequate ☐	
7. Notification of modifications to plant design	Agreed ☐	Not agreed ☐	

J. BIOLOGICAL SAFETY OFFICER

1. Training, experience and responsibilities	Adequate ☐	Inadequate ☐
2. Record system	Yes ☐	No ☐
3. Emergency procedure	Satisfactory ☐	Not satisfactory ☐
4. Deputy	Yes ☐	No ☐

K. MEDICAL OFFICER

1. Arrangements for helath monitoring	Adequate ☐	Inadequate ☐
2. GP/MO liaison	Yes ☐	No ☐
3. Health cards	Yes ☐	No ☐
4. Serum stored	Yes ☐	No ☐

Additional Comments

L. LOCAL SAFETY COMMITTEE

1. Properly constituted Yes ☐ No ☐
2. Regular meetings

M. INSPECTIONS BY OTHER BODIES

1. HSE Yes ☐ No ☐
2. Committee on Safety of Medicines Yes ☐ No ☐
3. Other (if Yes please specify) Yes ☐ No ☐

GENENTECH, INC.
460 POINT SAN BRUNO BOULEVARD
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(415) 952-0123 TWX: 9103717168

6 February 1980

Dr. Donald S. Fredrickson
Director, National Institutes of Health
Room 124, Building 1
National Institutes of Health
Bethesda, MD 20014

Dear Dr. Fredrickson:

Re: Proposed Part VII - Physical containment guidelines for large scale uses of organisms containing recombinant DNA molecules as noted in 44 FED. REG. 63074 (Nov. 1, 1979).

We are pleased to submit our comments on the proposed physical Guidelines for the large scale fermentation of organisms containing recombinant DNA molecules. Genentech has made two separate requests for approval for large scale developmental production of such microorganisms under existing Guidelines. As we anticipate continually making further future requests for new plasmids, Genentech is concerned that the Guidelines be not only effective from a safety aspect but also be practical in daily operation.

Our detailed comments on specific sections of the proposed Part VII Guidelines are attached. From a broader perspective, we offer the following suggestions for consideration.

o 1. The proposed Guidelines for large scale recombinant fermentations are an extension of the existing Laboratory Guidelines and continue the misconception that larger volumes imply larger risks.

Discussion: Large scale fermentations a priori present no more potential risk than smaller scale laboratory fermentations of recombinant organisms. In fact, one 400 liter fermentation in a well designed and operated stainless steel fermentor is much safer than using one glass four-liter flask on a shaker platform.

o 2. The P3-LS specifications should be equal to or less stringent than the existing laboratory P3 Guidelines.

Discussion: The P3-LS Level proposed Guidelines require many containment specifications and are accordingly significantly more demanding than existing laboratory P3 Guidelines. If anything, the P3-LS specifications should be equal or less stringent than laboratory P3 Guidelines as the written exception request to NIH to exceed 10 liters of culture requires that the DNA recombinants be "rigorously characterized and shown to be free of harmful genes". Typical P3 laboratory research with a wide variety of new uncharacterized plasmids has significantly higher potential risk without this rigorous characterization and establishment of the absence of harmful sequences. In addition, most P2 and P3 large scale fermentations are not experiments but are repetitive production operations using the same characterized culture in a standardized routine protocol with specific equipment, validated testing procedures and operating protocols which have been reviewed by the RAC and approved by the Director of NIH.

o 3. The large scale Guidelines should describe the specific confinement results to be achieved and not the means to this result.

Discussion: In several sections, the proposed large scale specifications describe the means to achieve a certain result rather than specifying the desired result. For example, section VII-C-3-k would require that all exhaust air from production equipment be HEPA filtered prior to discharge to the outdoors. The specification should rather require that exhaust air released to the outdoors from such aerosol generating equipment not contain viable recombinant organisms. The result could be achieved by a number of means that may be more practical for a particular installation, e.g., air incineration.

o 4. Large scale containment should be accomplished by the steel fermentor itself and not by a separate room around the fermentor(s).

Discussion: Insignificant attention has been directed to the excellent containment characteristics of modern stainless steel fermentors. With a well designed and tested stainless steel fermentor having contained sampling and treated exhaust air systems there is no need for a special fermentor room or even for curbing to be built around the fermentor (to contain the total volume of broth from a fermentor). Such requirements are extravagant and impractical for fermentors containing large amounts of culture medium (such as the greater than 10,000-L production batches) which will be required in the near future to bring the products of recombinant DNA technology to the public. In my ten years of extensive fermentation experience with both pilot plant and four large fermentor production facilities, I have never seen or heard of a fermentor rupturing or losing a significant amount of broth containing microorganisms. Indeed, in the case of any significant but highly improbable leak, the contents of steel fermentors can be rapidly disinfected by in-place chemical sterilization, e.g., the immediate addition of on-line formaldehyde solution.

o 5. The long time lag between preparation of large scale exception requests and receipt of approval by the Director of NIH for changes to plasmids for previously approved polypeptide products and equipment/protocols causes delays in the delivery of product benefits to the public, increases product costs and adds nothing to the safety considerations. The local IBC should be authorized to approve such requests for changes to plasmids.

Discussion: To shorten the significant time lag between beginning the preparation of written 10-liter culture exception requests, the subsequent approval by the local IBC, then submission to the RAC which only meets quarterly and final receipt of written approval by the Director of NIH, we suggest that the local IBC be permitted to authorize such work above 10 liters when the particular fermentation equipment, operating procedures, and polypeptide products (if any) of the recombinant organism have been previously approved by the Director for the same containment levels as requested in the new exception request. For example, Genentech has been approved by the Director for the large scale fermentation of specific EK1 host vector systems in a P2 laboratory with certain fermentors. We have now made several changes to the plasmids to increase yields of the same polypeptide products. The new recombinant DNAs have been well characterized and shown to be free of harmful sequences. The host vector systems will be grown in the same fermentor systems with operating protocols previously approved by the Director. However, even after approval by the local IBC, it will be necessary to submit the exception requests for the new plasmids for the same products to the Director via the RAC. This procedure not only dilutes the busy RAC agenda but introduces a two to four month delay in every product developmental schedule for each modified plasmid for the same products with no gain in safety. In summary, we request that new large scale culture requests which are modifications of approved submissions, particularly at the P1 HV1 and P2 HV1 levels, only require local IBC approval with written notification of ORDA in cases where the fermentors, polypeptide products, and protocol have already been approved by the Director in a previous submission.

We believe that the RAC and its working group which drafted the proposed large scale Guidelines have made a sincere and thoughtful effort to provide a list of safe specifications and procedures. However, some of the requirements add little to the desire for safety from potential risk and by extrapolating laboratory Guidelines, are quite impractical for large scale production of beneficial products from recombinant microorganisms. The appointment of an advisory group having extensive design and operating experience with large scale fermentation equipment would be highly desirable as we move further into large scale development and production with recombinant cultures.

Sincerely,

Brian T. Sheehan

Brian T. Sheehan, Ph.D.
Vice President, Manufacturing

GENENTECH, INC.

COMMENTS ON PROPOSED PART VII GUIDELINES -- LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

LARGE SCALE -P2 LEVEL

Page 3-VII-B-1-g: We believe that this requirement is too extreme for the P2-LS level. Laboratory coats should be adequate. Operating staff most frequently leave the area. We recommend use of the Laboratory Guidelines II-B-2-a-(12) specification.

Page 3-VII-B-1-h: The phrase "except as necessary for access" should be inserted following the word "closed."

Page 3-VII-B-1-m: The phrase "or disinfected by validated procedures" should be inserted following the word "autoclaved."

Page 4-VII-B-2-a: The phrase "HEPA filter" should be replaced by "filter(s) capable of sterilizing or equivalent procedure." This is a case of specifying a means rather than a desired objective. HEPA filters may not be the most practical means of accomplishing the objective in a particular installation.

Page 5-VII-B-2-b-(4): The word "disassembly" should be deleted as it does not apply to large-scale industrial fermentors, but only to small table-top fermentors. Large industrial or pilot plant stainless steel fermentors are cleaned and steam sterilized in place with no disassembly.

Page 6-VII-B-3-b: The requirement for "curb or similar architectural feature" is highly impractical and unnecessary. Steel fermentors do not rupture, explode, or develop huge leaks. The probability of such a massive spill is so low that it holds exceedingly low risk. To require such a curbing would require extensive renovation of existing facilities, would be impractical to work around and add almost nothing to the physical containment features. In the event of a highly unlikely failure of two valves in series, the contents of a fermentor can be rapidly disinfected by in-place chemical sterilization as needed. To safeguard against the spreading of a small leak such as during sampling or harvesting of the batch, we recommend adding the phrase "or 500 liters of broth, whichever is smaller" after the words "total liquid volume."

LARGE SCALE P3 LEVEL

Page 6-VII-C-1-b: This requirement for changing of personal clothing and shoes is almost the full P4 specification except for the deletion of the requirement for a change of undergarments and a shower each time an individual leaves the production area. In actual use, the operating staff in the production area would spend at least 10% of their work day frequently changing their clothing and shoes. Of more concern, the proposal is an excessively stringent specification and adds little to the lowering of risk. The existing laboratory P3 specification, II-B-3-a-(13), is more than adequate if it can be broadened to include jump suits which would be removed before leaving the production area. Head covers in the hot production environment should also be deleted as they are also not required for laboratory P3 work. The requirements for personnel doing large scale P3 work should be no more stringent than laboratory P3 research, as the large scale P3 cultures must be previously approved by the RAC and the Director of NIH in addition to being "rigorously characterized and are shown to be free of harmful genes." This is not true for laboratory P3 research which is potentially a higher risk.

Page 7-VII-C-1-d: Although we have no intention of having persons under 18 years of age in the P3 production area, why was the age specification increased from 16 years to 18 years old? The laboratory P4 minimum age is 18 year old compared to 16 years for laboratory P3 research. Once again, the erroneous implicaton is that large scale P3 production is potentially more hazardous than laboratory P3 research.

Page 8-VII-C-1-k: The phrase "except as necessary for access" should be inserted following the word "closed."

Page 8-VII-C-1-o: We suggest that the phrase "validated procedures for inactivation of the organisms have been employed" be substituted for "inactivation of the organisms has been demonstrated."

Page 8-VII-C-1-p: Insert the phrase "or disinfected by validated procedures" after the word "autoclaved."

Page 9-VII-C-2-a: The specific requirement for HEPA filters should be modified to "filters capable of sterilization or equivalent procedure" such as incineration.

Page 10-VII-C-2-b-(4): The word "disassembly" should be deleted per our comment for Page 5-VII-B-2-b-(4).

Page 11-VII-C-3-c: This requirement for complete sealing of the production area is a P4 specification and is unnecessarily stringent. The viable cells are totally contained in the fermentor. Rather, attention should be directed to a safe closed sampling system. We recommend this entire requirement be deleted.

Page 11-VII-C-3-g: The phrases "shall be provided within the production area" should be replaced by "shall be available to the production area."

Page 11-VII-C-3-h: The requirement for curbs or similar features to contain the total tank contents as also stated for the proposed LS-P2 proposal is excessive, adds little to lower risk and operationally is highly impractical. Please see our comments for Page 6-VII-B-3-b to only contain 500 liters or less of broth by curbing or similar feature.

Page 12-VII-C-3-k: Only production equipment which produces aerosols containing viable recombinant organisms should be treated by passage through filters capable of sterilizing. Therefore, the first sentence should be replaced as follows: "The exhaust air from biological safety cabinets and other containment units, as well as exhaust gas containing viable recombinant organisms from aerosol generating equipment shall be treated by passage through a filter(s) capable of sterilizing or equivalent procedure." The requirement for a separate air treatment device to have a separate individual exhaust blower would require the roof of the production area to sprout many individual blowers, an expensive and inefficient mode of operation. We recommend that the last word, "area", be followed by the phrase, "or designed as an integral part of the air handling system." This would permit some manifolding of the system.

THE UNIVERSITY OF ROCHESTER
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Frank E. Young, M.D., Ph.D.
Dean of School of Medicine & Dentistry
Director of Medical Center
275-3407

February 19, 1980

Elizabeth Milewski, Ph.D.
Scientist Administrator
Office of Recombinant DNA Activities
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Milewski:

Thank you very much for your photocopies of comments in response to the request for public comment on draft standards for large-scale research and production. The comments clearly fall into two categories. Category one represents those that are detail analyses of the specific language. Here I believe we need to rely on Emmett Barkley's digest of comments and synthesis of conflicting views. Particularly relevant will be the comments from Upjohn and Eli Lilly. In this arena the major concern appears to be with the type of consideration given to the scale-ups in relation to research laboratory procedures. As I have reviewed this again, I believe that the question of the apron versus a holding tank for liquid that is spilled from a fermenter is an important issue and one that we must discuss further. The rest of the comments are clearly detail and can be harmonized quite readily with the guidelines.

A second concern relates to the P1-LS category that was not included in our original write-ups. This was an oversight. We should determine whether those organisms, in which there is merely self-cloning, fall under this guideline at all or are totally exempt. My own feeling would be that the exempt experiments should be exempt for scale-up as well with the exception that informational MUA's are submitted, that is, the project be registered. This would enable P1-LS-1 to be used for those organisms which are exchanges and might require a little more surveillance.

The Gmag comments seem to indicate that inspection of the plant is an important part of the process. We have rejected that from our own guidelines, but we may wish to include it in the future if large-scale operations become more common. Clearly, a site visit is one of the best ways for determining the extent of containment.

Thank you for sending me this information.

Sincerely,

FEY:eb

Department for Professional Employees, AFL-CIO
815 16th Street, N.W., Washington, D.C. 20006 Phone 202/638-0320

May 22, 1980

Director
Office of Recombinant DNA Activities
Building 31, Room 4A52
National Institutes of Health
Bethesda, Maryland 20205

Sir:

This is in response to your notice in the Federal Register, April 30, 1980, pages 28904ff, related to the next meeting of the Recombinant DNA Advisory Committee (RAC) on June 5 and 6.

We strongly object to proposal 18, which would amend the Guidelines to require industrial members on the RAC, and other things. The RAC has already stepped well beyond its authority and mandate by offering review and approval of industrial pilot plant and production processes using organisms produced through recombinant DNA techniques. This gross error would only be compounded by any attempt to add industrial expertise to the committee beyond that of industrial consultants who may already be members.

The function of the RAC is to review research involving recombinant DNA. Regulations of the use of the results of this research in industrial production is properly and legally the duty of the appropriate regulatory agencies. Whether or not those agencies are currently in a position to properly carry out their regulatory functions in this area is a question for those agencies, and not the RAC, to answer.

We would certainly be concerned with anything which had the effect of circumventing the intent of Congress. Our members look to the legitimate regulatory agencies for protection, and not to an agency whose primary function is to fund scientific research.

Yours truly,

Dennis Chamot, Ph.D.
Assistant Director

DC:gl
cc: Senator Adlai E. Stevenson
George Taylor, AFL-CIO

Industrial Union Department AFL-CIO

815 16th Street N.W.
Washington, D.C. 20006
202/393-5581

Howard D. Samuel, President
Elmer Chatak, Secretary-Treasurer

May 23, 1980

Director
Office of Recombinant DNA Activities
Building 31, Room 4A52
National Institutes of Health
Bethesda, Maryland 20205

RE: FR, April 30, RAC

Sir:

We object to proposal 18, which would amend the Guidelines for recombinant DNA research to permit industrial members on the RAC. RAC has exceeded its authority by offering review and approval of industrial pilot plant and production processes. Any attempt to add industrial expertise to the committee, beyond that of industrial consultants who may already be members, is to convert the committee to a self-appointed consensus standards organization. Such a conversion is not advisable and, if it were to occur, it could be in violation of the current OMB circular on such bodies.

The function of the RAC should continue to review research. Regulation of industrial production is the function of regulatory agencies. By this digression from the mandated functions of NIH, the nation's health research program is once again diluted by activities not within NIH competency.

Respectfully Submitted,

Sheldon W. Samuels, Director
Health, Safety and Environment

SWS:pcs
opeiu#2afl-cio
cc: Dr. Chamot
    Dr. Frederickson, Director, NIH
    Dr. Bingham, Assistant Secretary for OSHA
    Dr. Robbins, Director, NIOSH

Vice Presidents

Shelley Appleton • Kenneth Blaylock • Peter Bommarito • Dominick D'Ambrosio • David J. Fitzmaurice • Wayne E. Glenn • Alvin L.
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Albert Shanker • Jacob Sheinkman • Carl W. Studenroth • J. C. Turner • Shannon Wall • Martin J. Ward • Glenn E. Watts • William W.

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG
Willson Professor of Biochemistry

July 29, 1980

Dr. Maxine Singer
Bldg. 37, Room 4A-01
National Cancer Institute
Bethesda, MD 20205

Dear Maxine:

I am writing to you about your proposal to modify the administrative requirements now demanded by the NIH guidelines for researchers using recombinant DNA technologies. I applaud the initiative you have made and will do what I can to persuade RAC and others of the necessity to move to the point or at least the direction you suggested. Frankly, as you know, I favor even more substantial eradications in the guidelines, but, perhaps, it is best to begin as you have suggested.

There is a point which I did not see addressed, although it may be buried somewhere in the pages of Section III and IV that you marked up. Investigators submitting a new research grant proposal, on an annual non-competing renewal or a competing renewal, must now submit approved MUAs for experiments to be done during the ongoing years. The annual application to the IBCs and ORDA for renewal of ongoing MUAs is in my view one of the more frustrating and useless exercises in the present system. Would your proposed modifications remove the necessity to submit accompanying MUAs from the grants review process? Could you incorporate that into your proposal? I hope so.

I particularly support the admonition made in the penultimate paragraph of your recommendation. I believe the NIH guidelines have served their purpose. They, and the process used to produce them, educated scientists to the anxieties that were raised and developed advice on how to perform the work safely. There is now a widespread acceptance and voluntary compliance of these recommendations and I see no reason why that would not continue without all the red tape. The time has come to reconsider the costs and benefits of the RAC-ORDA establishment.

With best wishes and regards,

Sincerely,

Paul

Paul Berg

PB/hk

August 22, 1980

Dr. Donald S. Frederickson
Director
National Institutes of Health
Bethesda, Maryland 20205

Reference: Request for an exception to prohibition I-D-4 in order to field test corn genes put into corn with an E. coli or S. cerevisiae vector. 45 Federal Register 28907 (item 10), April 30, 1980.

Dear Dr. Frederickson:

The Environmental Defense Fund (EDF) hereby requests the National Institutes of Health (NIH) to prepare an environmental impact statement (EIS) on the above referenced proposal to field test corn plants (Zea mays) into which corn recombinant DNA has been added using an E. coli or S. cerevisiae vector.

On April 30, 1980 NIH noticed in the Federal Register the proposed experiment by Dr. Ronald Davis of Stanford University Medical Center to obtain an exception to the prohibition of deliberate release into the environment of any organisms containing recombinant DNA.

A final decision on this proposal was deferred, pending receipt of additional information (45 Federal Register 50524, July 29, 1980). The preparation of an EIS could substantially aid NIH in obtaining as much additional information as a prjeect of this significance should have.

We wish to emphasize that we do not object either to the experiment per se or to the desirable goal of increasing protein in corn. As George Beadle wrote in the Smithsonian Magazine recently, "Corn is the most efficient of all cereals in converting solar energy, carbon dioxide, water and mineral nutrients into foodstuff. It is now the world's third most important food crop. . ."

For American agriculture, the story of hybrid corn is well known as "one of the great contributions of science

Environmental Defense Fund, 1525 18th Street NW, Washington, DC 20036 (202) 833-1484
OFFICES IN: NEW YORK, NY (NATIONAL HEADQUARTERS); WASHINGTON, DC; BERKELEY, CA; DENVER, CO

and technology to the feeding of an expanding population."*/ Corn now can be planted closer, can use fertilizer more efficiently, can be planted earlier, cultivated more easily, and harvested mechanically. Obviously increasing the protein content in corn would represent yet another remarkable breakthrough.

The proposed experiment should be scrutinized by a broad spectrum of scientists knowledgeable or interested in corn because the acreages planted in corn stretch the continent, because the corn now planted is nearly uniform throughout the country, and because the 1970 corn blight epidemic demonstrated how a mutant strain of H. maydis made "a liability out of a technology that had been devised to improve the efficiency of the nation's agriculture." **/

The corn blight of 1970 is well known. "The losses in 1970 wiped out, for that year at least, some of the gain in efficiency in corn production acquired so laboriously during 50 years of research and its technological application." ***/

We cannot, of course, hope to foresee all problems, but we have a responsibility to minimize potential negative impacts of technologies going awry, by trying to anticipate secondary effects from proposed technologies ahead of time.

The corn agoecosystem is one of the world's most intensive agricultural production centers. For example, over 95% of corn acreage in the Corn Belt is treated with herbicides for weed control. ****/ Among the herbicides in high use on this acreage is atrazine. The enclosed study, "Mutagenicity of atrazine: a maize-microbe bioassay," was published in 1976 and discusses the possibility that the corn plant converts chemicals into mutagenic agents. There have also been studies indicating that 2,4-D increases insect and pathogen pests on corn, which in turn requires heavier applications of insecticides.

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*/ National Academy of Sciences, Genetic Vulnerability of Major Crops, 1972 at 6.

**/ Id at 7-9.

***/ Id at 9

****/ Office of Science and Technology Report, Pest Management Strategies for Food Production in the Central Corn Belt, October, 1979 at 5.

The result is a multitude of different chemicals being applied to corn, with many interactions not yet studied or understood. Some of the most heavily used pesticides could be tested in the laboratory for synergisitic effects in and on corn plants before open field testing of the proposed experiment.

We wish to praise the scientist proposing this experiment for his imagination and persistence in the face of difficulty in obtaining funds and approvals. He should not read this request for an EIS as an obstacle, or an objection, to his basic goal or the desired eventual field tests. Our awareness of some of the complexities of agricultural technologies and the nature of corn production in the United States, as well as the significance of corn for the world's food needs obligates us to ask for very specific and broad scrutiny of the proposed project before field testing. In addition to academic scientists, relevant federal and state agencies, and private organizations should be made aware of the proposal and be able to submit comment.

An initial list of scientists who could be asked to add substantive evaluation of this proposed project is enclosed.

We would appreciate a response to this request.

Sincerely yours,

Maureen K. Hinkle

Maureen K. Hinkle
Pesticides Monitor

cc: William J. Gartland, Jr.
Jane Setlow
Sue A. Tolin

LIST OF SCIENTISTS

Peter Day
Director
Plant Breeding Institute
Trumpington
Cambridge, UK CB 2 2LQ

Professor James M. Gentile
Department of Human Genetics
Yale University School of Medicine
New Haven, Connecticut 06510

Dr. F. Grossman
and Dr. J.A. Pfister
Institute fur Phytomedizin
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Postfach 106
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Dr. R.E. Gunn
Kansas State University
Manhattan, Kansas

formerly at the Plant Breeding Institute in England. Currently at Kansas State University

Dr. I.N. Oka
Dept. Entomology
Cornell University
Ithaca, NY 14850

Professor Peter Peterson
Department of Plant Genetics
Iowa State University
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Professor David Pimentel
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Dr. Michael J. Plewa
Department of Agronomy
College of Agriculture
University of Illinois
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Dan Winkelmann
Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)
Apartado Postal 6-641,
Mexico 6, D.F., Mexico

THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY

725 N. WOLFE STREET
BALTIMORE, MARYLAND 21205

September 3, 1980

Dr. William Gartland
Chief, Office of Recombinant DNA Activities
National Institutes of Health
Bldg. 31, Rm. 4A-52
Bethesda, MD 20205

Dear Dr. Gartland:

I would like to comment on the proposed changes in the NIH guidelines suggested by Dr. Maxine Singer and published in the Federal Register of Thursday, August 21, 1980. I strongly support the proposed changes. In my opinion they represent a natural and justified evolution in the responsibility of local biosafety committees. At Johns Hopkins University the committee functions extraordinarily well, and its role in the review of proposals is highly respected. Elimination of central review at NIH for experiments classified in the Guidelines will save a great deal of time for both the investigator and the reviewers at NIH. I believe the experience of the past six years justifies a change toward an administrative arrangement long successfully practiced in the handling of proven microbial pathogens, namely national guidelines enforced by local biosafety committees. Dr. Singer's proposals are a step in this direction.

Sincerely,

Daniel Nathans

Daniel Nathans
Professor and Director
Department of Microbiology

DN/lc

McARDLE LABORATORY
FOR CANCER RESEARCH
DEPARTMENT OF ONCOLOGY
MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN

September 3, 1980

Dr. William J. Gartland
Office of the Recombinant DNA Activities
N.I.H. (Building 31 - Room 4A52)
Bethesda, MD 20014

Dear Bill:

This letter is in support of Dr. Maxine Singer's proposal [Feder. Reg. 45 (1980) No. 164, p. 55929, section 8] to change and eliminate most of the administrative requirements specified by the NIH Guidelines. Certainly, the absence of any practically-significant risks does not warrant the present cumbersome bureaucracy, paperwork, and wasted time. Dr. Singer's proposal is a step in the right direction, with the next step being the suspension of the entire regulatory machinery spawn by the NIH Guidelines, until such time when any significant risks should materialize and be proven. The present regulatory aspects of NIH are of no benefit to science or Society, while sapping our resources and good will, and creating new dangers of their own, as per the enclosed article in TIBS.

I hope that the changes proposed by Dr. Singer will be promptly instituted.

With the best personal regards,

Sincerely yours,

Waclaw Szybalski
Professor of Oncology

WS:ms

cc: Dr. Maxine Singer

450 North Randall Avenue, Madison, Wisconsin 53706 608-262-2177

STANFORD UNIVERSITY MEDICAL CENTER
DEPARTMENT OF GENETICS

September 5, 1980

Dr. William Gartland
Chief Officer, Recombinant DNA Activities
National Institutes of Health
Building 31, Room 4A-52
Bethesda, MD 20205

Dear Dr. Gartland:

I am writing in strong support of the changes in registration requirements for recombinant DNA experiments proposed by Dr. Maxine Singer and published in the Federal Register of August 21, 1980.

I believe that the changes proposed by Dr. Singer are consistent with the level of our current understanding and knowledge about recombinant DNA research. Institutional biosafety committees clearly seem able to operate as independent review groups, and the laboratory practices set forth in the Guidelines have now become part of the standard operating procedures of laboratories working in the field. In other fields of biological research, safety procedures are implemented entirely at the local level, even when known biohazards are involved. It is no longer necessary to have a unique multi-level system in an area where seven years of extensive experience has shown the absence of a unique hazard. The proposed changes would do much to place things in proper perspective.

I urge the approval of Dr. Singer's proposal.

Sincerely yours,

Stanley N. Cohen

Stanley N. Cohen
Professor

SC:TA

DEPARTMENT OF GENETICS, STANFORD UNIVERSITY SCHOOL OF MEDICINE, STANFORD, CALIFORNIA 94305 • (415) 497-5052

THE JOHNS HOPKINS HOSPITAL
BALTIMORE, MARYLAND 21205
Department of Pediatrics

9 September 1980

Dr. William Gartland
Chief, Office of Recombinant DNA Activities
National Institutes of Health
Bldg. 31, Room 4A-52
Bethesda, Maryland 20205

Dear Dr. Gartland:

This letter is written to support Dr. Singer's proposal to streamline recombinant DNA research guidelines by eliminating NIH review, registration, and approval for all experiments assigned containment conditions. The proposal makes great sense since individual investigators will still be responsible for notifying IBCs of their activities.

Sincerely yours,

Haig H. Kazazian, Jr., M.D.
Professor of Pediatrics

HHK/bme

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF BIOPHYSICS AND THEORETICAL BIOLOGY
CUMMINGS LIFE SCIENCE CENTER
920 EAST 58TH STREET
CHICAGO · ILLINOIS 60637

9 September 1980

Director, Office of Recombinant DNA Activities
Building 31, Room 4A52
National Institutes of Health
Bethesda, Md. 20205

Dear Sir:

I urge adoption by the RAC of the revisions of the Recombinant DNA Guidelines proposed by Dr. Maxine Singer, appearing on pp. 55926-55928 of the Federal Register for August 21, 1980. These revisions will eliminate a great deal of unnecessary paperwork and expense, shift responsibility appropriately to local IBC's, and allow ORDA to deal with substantive issues more effectively.

Sincerely yours,

Robert Haselkorn
F.L. Pritzker Professor and Chairman

RH/bpm

UNIVERSITY OF WISCONSIN-MADISON

DEPARTMENT OF HORTICULTURE

1575 Linden Drive
Madison, Wisconsin 53706
608-262-6503

Dr. William J. Gartland
Office of the Recombinant DNA Activities
N.I.H. (Building 31-Room 4A52
Bethesda, MD 20014

September 10, 1980

Dear Dr. Gartland:

The Federal Register of August 21st 1980 (Vol. 45, No. 164, pages 55926-55928) contains suggestions by Dr. Maxine Singer for major changes in the registration requirements for recombinant DNA research. I strongly endorse the proposed changes because I believe that the risks of this research are now clearly minimal and because the bureaucracy and consequent wasted time are truly detrimental to progress in an area that promises to be of great benefit to mankind. Continued enforcement of unneccessary regulations can only serve to perpetuate doubts in the minds of the layman about research that he cannot conceptualize, and provides copious ammunition to the many scandalmongers who profit by arousing public concern unneccessarily.

Yours sincerely,

Timothy C. Hall
Professor

TCH:cz

University of Wisconsin-Madison provides equal opportunities for admission and employment.

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY
STANFORD UNIVERSITY SCHOOL OF MEDICINE

Area Code
497-

September 10, 1980

Dr. William Gartland
Chief, Office of Recombinant DNA Activities
National Institutes of Health
Bldg. 31, Rm. 4A-52
Bethesda, MD 20205

Dear Dr. Gartland,

I am writing you in support of Dr. Maxine Singer's recent proposal to ORDA to eliminate the central NIH review, registration and approval of recombinant DNA experiments explicitly assigned containment conditions in the NIH guidelines. I agree with her that the function of review, registration, and monitoring of research workers falling into categories explicitly mentioned in the Guidelines can be effectively administered by the local Institutional Biosafety Committees. This would reduce the workload for ORDA allowing it to spend more time determining policy and revising the guidelines according to requests or to new data. It would simultaneously ease the bureaucratic work load for the researcher without jeopardizing the effectiveness of the Guidelines. Not only are the procedures described in the Guidelines now standard practise among most laboratories, as pointed out by Dr. Singer, but I feel that the local Institutional Biosafety Committees are much better position to evaluate compliance with the Guidelines. Because of their past experience with the Guidelines I feel that these committees are ideally suited to take over this important administrative role.

Sincerely,

Douglas L. Brutlag

THE ROCKEFELLER UNIVERSITY
1230 YORK AVENUE · NEW YORK, NEW YORK 10021

September 10, 1980

Dr. Donald S. Fredrickson, Director
Office of Recombinant DNA activities
Bldg. 31, Room 4A52
National Institutes of Health
Bethesda, MD 20205

Dear Don:

Maxine Singer's initiative (Fed. Reg., Aug. 21, 1980) to have the NIH Guidelines fall primarily under local control is both wise and timely. Decisions about scientific research are best left to the investigators both for efficiency and as a matter of fundamental principle. Only in exigency should this ideal be violated. Perhaps we've passed through such an occasion with R-DNA research. However, it is now the time to return to a more normal situation. Since work with recombinant DNA has proven to be very safe and very important, dual review at the national and local levels serves no good purpose.

As one involved from the beginning in the R-DNA issue, I've always felt that the "Guidelines" should be guidelines; they turned into regulations. With the proposed change they will revert and also the unfortunate quasi-regulatory role that the NIH has had to play will begin to disappear.

I can think of no reason for not dispensing with the MUAs. I hope that you will be able to institute this welcome change.

Yours sincerely,

Norton

Norton D. Zinder

THE JOHNS HOPKINS UNIVERSITY
SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY

725 N. WOLFE STREET
BALTIMORE, MARYLAND 21205

September 11, 1980

Dr. William Gartland
Chief, Office of Recombinant DNA
   Activities
National Institutes of Health
Bldg. 31, Rm. 4A-52
Bethesda, MD 20205

Dear Dr. Gartland:

I have recently learned that Dr. Maxine Singer has written to you a letter proposing major changes in the administration of the NIH Guidelines for Research Involving Recombinant DNA. I take this opportunity to express my strong support for the changes as outlined in the Federal Register/Vol. 45, No. 164/Thursday, August 21, 1980/Notices (page 55926). These changes would significantly expedite recombinant DNA work without altering safety requirements.

Sincerely yours,

HOSmith

Hamilton O. Smith

HOS:mk

ROCHE INSTITUTE OF MOLECULAR BIOLOGY
Nutley, New Jersey 07110

September 11, 1980

Dr. William Gartland
Office of Recombinant DNA
National Institutes of Health
Bethesda, Md. 20205

Dear Bill:

I am writing in support of Dr. Maxine Singer's proposed changes in registration requirements for recombinant DNA experiments as published in the Federal Register, Vol. 45, August 21, 1980 (Item #8).

These changes delegate primary responsibility to the Institution and the investigator as is reasonable and appropriate.

In addition, the proposed changes in administrative requirements should tend to re-establish the "Guidelines" as guidelines and not regulations, a move in keeping with their original intent.

Yours truly,

A. M. Skalka, Ph.D.
Department of Cell Biology

AMS:pcp

STANFORD UNIVERSITY, STANFORD, CALIFORNIA 94305
DEPARTMENT OF BIOLOGICAL SCIENCES

CHARLES YANOFSKY
MORRIS HERZSTEIN PROFESSOR
OF BIOLOGY

September 12, 1980

Dr. William Gartland, Chief
Office of Recombinant DNA Activities
National Institute of Health
Building 31, Room 4A-52
Bethesda, MD 20205

Dear Dr. Gartland,

I have read Dr. Maxine Singer's letter of August 4th to you (Federal Register Vol 45; No. 164 page 55926, August 21, 1980) and strongly approve of her suggestions. At this point in the history of recombinant DNA research it makes sense to have proposed projects that are covered in the Guidelines administered by the local IBC's alone, thereby relieving your office and every research institution of the burden of processing MUAs.

I hope Dr. Singer's proposals will be given very serious consideration.

Sincerely,

Charles Yanofsky

Charles Yanofsky

CY/sbr

University of Wisconsin Madison

LABORATORY OF GENETICS
Genetics Building
445 Henry Mall
Madison, Wisconsin 53706

September 12, 1980

Dr. William J. Gartland
Office of the Recombinant DNA Activities
N.I.H. (Building 31 - Room 4A52)
Bethesda, MD 20014

Dear Bill,

I support heartily the elimination of paperwork advocated by Dr. Singer [Feder. Reg. 45 (1980) No. 164, p. 55929, section 8].

Yours truly,

Frederick R. Blattner

Frederick R. Blattner
Associate Professor of Genetics

FRB/wf

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY
STANFORD UNIVERSITY SCHOOL OF MEDICINE

Area Code 415
497-6161

September 12, 1980

Dr. William Gartland
Office of Recombinant DNA Research
National Insitute of Health
Bethesda , Maryland 20014

Dear Bill:

I understand that you have already seen my letter to Maxine Singer regarding her proposal to eliminate the memoranda of understanding (MUAs) from the administrative practices associated with recombinant DNA research. Nevertheless, let me reiterate my strong support for enacting her recommendation. I think the IBC mechanism has proven its effectiveness in interpreting and enforcing the letter and intent of the Guidelines governing recombinant DNA research. I do not see how the practices of submission, obtaining and recording, etc., of MUAs, particularly the requirements for annual submissions of MUAs for each continuing NIH grant or for applications for new grants, contributes to the safety of research. An updated statement of work in progress from each investigator to the IBCs would be significantly simpler to administer and would ensure as much real safety as the present system. The Guidelines would then serve as guidelines to the IBCs as to what is permissible and what is not. The Guidelines could have the same function as the CDC guidelines in that they would assign different types of experiments to various categories of containment or handling practices and rely on IBCs (or Health and Safety Committees) to monitor compliance.

I hope that some movement to loosen the reins on research with recombinant DNA techniques can be implemented as quickly as possible.

Sincerely,

Paul

PB/hk

SCHERING CORPORATION

60 ORANGE STREET BLOOMFIELD, N. J. 07003

TELEPHONE: (201) 743-6000
TELEX: 138183
CABLES: SCHERING BLOOMFIELD, N. J.

September 18, 1980

Dr. W. J. Gartland
Director, ORDA
National Institute of Allergy
  and Infectious Diseases
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

The NIH Guidelines for Research Involving Recombinant DNA Molecules, issued on January 29, 1980, state on page 6749, Appendix E, that "P2 physical containment shall be used for DNA recombinants produced between members of the genera *Streptomyces* and *Micromonospora* except for those species which are known to be pathogenic for man, animals or plants." These two genera are part of the large group of *Actinomycetes* which produce therapeutically active compounds.

Although some members of *Actinomycetes* are pathogenic, most are non-pathogenic for man, animals or plants. Rather than limit the cloning experiments permissible at the P2 level to just non-pathogenic members of *Streptomyces* and *Micromonospora*, I wholeheartily support Dr. Dean Taylor's proposal to include all of the non-pathogenic members of the order *Actinomycetales* at this level of containment.

Sincerely,

Mark Finkelstein

Mark Finkelstein, Ph.D.
Molecular Biology Department

MF:ds

STANFORD UNIVERSITY MEDICAL CENTER

DEPARTMENT OF GENETICS

September 18, 1980

Dr. William J. Gartland, Jr.
Director
Office of Recombinant DNA Activities
Building 31, Room 4A-52
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

This letter is written in support of the request by Dr. Dean P. Taylor for reconsideration of the proposal that P2 physical containment be used for DNA recombinants produced between members of the *Actinomycetes* group except for those species which are known to be pathogenic for man, animals or plants.

It is well recognized that the vast majority of *Actinomycetes* are free-living soil organisms that pose no known hazard and are not pathogenic. It is reasonable that work with *Actinomycetes* other than *Streptomyces* and *Micromonospora* be carried out at the P2 level of containment except for a few organisms. Dr. Taylor proposes an appropriate way of dealing with those few organisms: they should be included in a list of organisms requiring higher level containment, in the same that other pathogenic species and genera are explicitly listed in the Guidelines.

I hope that the RAC will see fit to approve the proposed change in the Guidelines.

Sincerely yours,

Stanley N. Cohen

Stanley N. Cohen
Professor

SNC:ps

DEPARTMENT OF GENETICS, STANFORD UNIVERSITY SCHOOL OF MEDICINE, STANFORD, CALIFORNIA 94305 • (415)

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY
TANFORD UNIVERSITY SCHOOL OF MEDICINE

Area Code 415
497-6161

September 19, 1980

Dr. William Gartland, Chief
Office of Recombinant DNA Activities
National Institutes of Health
Building 31, Room 4A-52
Bethesda, Maryland 20205

Dear Dr. Gartland:

This letter concerns the NIH Guidelines for Research involving recombinant DNA. In the August 21st issue of the Federal Registry concerning recombinant DNA research, there was a proposal by Maxine Singer to eliminate the requirement for obtaining MUA's to carry on recombinant DNA experiments. I strongly support this proposal and feel that it is time for ORDA to turn these responsibilities over to the local IBC's. This would place more responsibilities on the local IBC's, where I think it belongs. It is essential that the bureaucracy surrounding recombinant DNA research be progressively decreased.

Sincerely,

Ronald W Davis

Ronald W. Davis
Professor of Biochemistry

RWD/ns

Yale University

SCHOOL OF MEDICINE
333 Cedar Street
P.O. Box 3333
Department of Human Genetics
New Haven, Connecticut 06510

September 22, 1980

Dr. William Gartland, Jr.
Chief, Office of Recombinant DNA Activities
National Institutes of Health
bldg. 31, Rm. 4A-52
Bethesda, Maryland 20205

Dear Bill:

This is to inform the R.A.C. that Yale's Institutional Biosafety Committee has voted unanimously to endorse Dr. Maxine Singer recommendation, as outlined in her letter to you of August 4, 1980.

We feel strongly that safety is solely a function of the effectiveness of the local institutional practices, on the part of the P.I.'s and the I.B.C., in complying with and enforcing the Guidelines. The submission of M.U.A.s adds nothing but expensive and time-consuming paperwork.

Sincerely yours,

Edward A. Adelberg

Edward A. Adelberg
Professor of Human Genetics
Chairman, Institutional
Biosafety Committee

EAA:nd

cc: Dr. Maxine Singer

STANFORD UNIVERSITY
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOLOGICAL SCIENCES

September 22, 1980

Dr. William Gartland, Chief
Office of Recombinant DNA Activities
National Institute of Health
Building 31, Room 4A-52
Bethesda, Maryland 20205

Dear Dr. Gartland:

I fully support in principle the proposals of Dr. Maxine Singer, as published in Federal Register 45, No. 164 (August 21, 1980), pages 55926-55928. Specifically, submission of MUA's to ORDA should no longer be required for grant proposals or ongoing grant support. Local IBC's should be authorized to review and approve experiments covered by the Guidelines, while ORDA would function mainly to interpret and clarify the Guidelines.

The present complex system of review on both local and Federal levels is no longer necessary. It is wasteful of time, effort, and money. It is, in fact, counterproductive because bureaucratic requirements seen by investigators to be clearly unnecessary lead to disrespect for regulations that should be respected. Institutional Biosafety Committees should, in my opinion, be fully capable of monitoring laboratory practices and containment levels specified by the Guidelines.

While I have not checked all minor details of the wording changes, the main changes proposed by Dr. Singer seem to me to be clearly justified by current knowledge. I expect that the time is not far off when federally mandated bureaucratic procedures related to Recombinant DNA can be completely eliminated.

Sincerely yours,

David D. Perkins

David D. Perkins
Professor of Biology

DDP:gm

BOARD OF PUBLIC AND SCIENTIFIC AFFAIRS

AMERICAN SOCIETY FOR MICROBIOLOGY

1913 I STREET, N.W.
WASHINGTON, D. C. 20006
TELEPHONE: CODE 202, 833-9

22 September 1980

Dr. Donald S. Fredrickson, Director
National Institutes of Health
9000 Rockville Pike
Bethesda, Maryland 20205

Dear Dr. Fredrickson:

The Committee on Genetic, Molecular and Systematic Microbiology of the Board of Public and Scientific Affairs of the American Society for Microbiology would like to comment on several issues for consideration at the Recombinant DNA Advisory Committee Meeting on September 25 and 26, 1980.

1. Item 7. We agree with the RAC proposal past in June 1980, to exclude regulatory functions dealing with industrial scale-up. We believe that the current mission of the RAC is appropriate and that its present constitution, representing scientific and public interests, is not well suited for a regulatory role. Other bodies of the federal government already have the mandate to carry out regulatory functions and should acquire necessary resources and expertise.

2. Item 8. At present, we do not believe that there are good reasons for changing the registration requirements. Although present procedures demand some extra paperwork, they permit centralized keeping of files which could be of significant use in reviewing the extent of work on recombinant DNA and its possible hazards. Somebody should know what is going on.

3. Item 11. We have long held the view that the RAC membership should include persons versed in scientific aspects of industrial microbiology. As with the rest of the Committee, they should be selected for the excellence of their qualifications. Such persons would complement the present technical expertise of RAC by providing knowledge in areas of fermentation technology and large-scale industrial applications. This expertise differs from that of safety engineers and is concerned with basic biological problems of large-scale technology. The Committee on Genetic, Molecular and Systematic Microbiology made recommendati for industrial representation on RAC in a letter to you dated 24 April 1980.

Sincerely yours,
M. Schaechter
Moselio Schaechter, Ph.D.
Chairman, Committee on Genetic,
Molecular and Systematic Microbiolog
C.D. Cox
C.D. Cox, Ph.D.
Chairman, Board of Public and
Scientific Affairs

U.S. Department of Labor

Assistant Secretary for
Occupational Safety and Health
Washington, D.C. 20210

SEP 24 1980

Dr. Donald Fredrickson
Director
National Institutes of Health
5600 Fishers Lane
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

As you know, both the NIH Recombination DNA Advisory Committee (RAC) and OSHA, among others, have been concerned with how the recombinant DNA industry should be monitored in an attempt to protect workers from any existing or potential hazards which may be peculiar to this type of activity. OSHA has been actively reviewing the ways in which we can fulfill our mandate in this regard.

In recognition of our need to develop a regulatory policy concerning recombinant DNA, representatives of OSHA and NIOSH have been attending the RAC meetings and participating in the meetings of the Interagency Advisory Committee on Recombinant DNA Research and of its Industrial Practices Subcommittee. In addition, NIOSH has undertaken a study of the industry and its potential hazards, as decribed in the attached memorandum, which will provide a basis upon which OSHA regulatory decisions in this area can be developed. We estimate that for completion of the NIOSH study and for implementation of OSHA's subsequent decisions, a period of approximately two years will be required.

I regret that the RAC wishes to terminate its reviews of engineering plans for proposed private-sector DNA operations. Because this would remove one of the existing procedures for promotion of biological safety in these operations, I would like to describe to you how OSHA proposes to deal with recombinant DNA workplaces during the interim period, until OSHA regulatory policy for this industry is developed.

Until formal regulatory decisions are made, OSHA will regulate recombinant DNA operations under its current authority, standards, and guidelines. Thus, OSHA will respond to complaints from workers, unions, or others about recombinant DNA hazards by reviewing the complaints and conducting inspections as warranted. In addition, inspections can also be initiated by OSHA without receipt of a specific complaint if the workplace selection is made in conformity with OSHA's current guidelines on general schedule inspections. If an inspection of a recombinant DNA facility is undertaken, OSHA compliance officers will, consistent with OSHA established practice,

look for violations of existing standards and of the general duty provision (Section 5(a)(1)) of the OSH Act which states, in part, that the employer shall furnish a place of employment free from recognized hazards that are likely to cause serious physical harm. Furthermore, during such an inspection, OSHA may choose to determine whether the employer is in compliance with the RAC recommendations and with applicable sections of the NIH guidelines. OSHA will inform the employer of any observed failures of such compliance and will determine what OSHA action should be taken to bring about worker protection from recombinant DNA hazards at that workplace.

We hope that these procedures by OSHA, coupled with the continued function of the RAC and continued voluntary compliance by industry with RAC recommendations, will provide for safety of the workers in this developing industry.

Sincerely,

Eula Bingham

Eula Bingham
Assistant Secretary
Occupational Safety and Health

STANFORD UNIVERSITY MEDICAL CENTER

DEPARTMENT OF GENETICS

October 9, 1980

Dr. Donald Frederickson
Director
National Institutes of Health
Building 1, Room 124
Bethesda, MD 20205

Dear Dr. Frederickson:

I understand from talking with Dr. William Gartland and others present at the last RAC meeting that an amendment to Action 916 offered by Dr. Richard Novick at the meeting would require the use of non-conjugative plasmids for the cloning of *B. subtilis* or *E. coli*- derived DNA in *Streptomyces* species. Since non-conjugative *Streptomyces* plasmids are not known to exist, and since an action approved previously by the Committee permitted cloning of the above DNA's in *S. coelicolor* under P2 conditions without specifying the nature of the vector, Dr. Novick's amendment would have the effect of reversing a previous decision by the RAC and increasing the level of containment required for experiments that have been permitted under P2 conditions for some time. Thus, the amendment is a "major action"; it does not seem appropriate that it be made a part of the Guidelines in the absence of a 30-day period providing an opportunity for workers in the field and others to offer comments on it.

It should also be noted that the concept of "conjugative" versus "non-conjugative" plasmids has been derived from enteric bacteria and may not apply to a wide variety of other bacterial species. For example, the mechanism for genetic exchange in *Streptomyces* species is not fully understood; it may be a consequence of hyphal fusion that permits transfer of plasmid and chromosomal genes. If this is true, it may never be possible to isolate a "non-conjugative" plasmid *per se* to meet the requirements specified by Dr. Novick's amendment.

Since all of the *Streptomyces* species that exchange genetic information with *S. coelicolor* are free-living non-pathogenic soil organisms --- and since the change in the Guidelines originally proposed under Action 916 involved genes from only non-pathogenic, well-characterized donor bacteria, I urge your approval of the original proposal without the amendment.

Sincerely yours,

Stanley N. Cohen

Stanley N. Cohen
Professor

SNC:ps

DEPARTMENT OF GENETICS, STANFORD UNIVERSITY SCHOOL OF MEDICINE, STANFORD, CALIFORNIA 94305 • (415) 497-5052

Research and Development

SK&F
a SmithKline comp

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cable SMITHKLINE PHILADELPH
telex 83-44

October 15, 1980

Dr. Donald S. Fredrickson
Director, National Institutes
of Health
Bethesda, MD 20205

Dear Dr. Fredrickson:

I would like to bring to your attention a change in the NIH Guidelines for Research involving recombinant DNA molecules. This change, in my opinion, is a major action and should not be approved without further consideration. The change to which I refer occurred on September 26, 1980 during consideration by the RAC of a proposed amendment of appendix E of the guidelines (see item 9 of the Federal Register, vol. 45, no. 164, Thursday, August 21, 1980, p. 55928).

The proposal was amended by Dr. Richard Novick so as to read "*Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K-12, or from *S. aureus* vectors that have been approved for use in *B. subtilis,* under P2 conditions using as vectors any non-conjugative plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms." The change from the published text, initiated by Dr. Novick, is underlined.

I would like to see this amended proposal published for public comment and put to a second vote at the next RAC meeting before you approve this action. This amended proposal failed to receive sufficient consideration of the following points. First of all, in no previous actions regarding *Streptomyces* species was any consideration given during the deliberations to establish levels of physical containment as to whether the vectors were capable of mediating exchange of genetic information. Therefore, this action by the RAC is more restrictive than previous actions and, in my opinion, is inconsistent with the intent of the RAC. In fact, experiments can be carried out at P2 levels of containment in which *B. subtilis, E. coli* K-12, or *S. aureus* vector DNA is cloned into *S. coelicolor* hosts using vectors capable of self-mediated exchange in accordance with Appendix E of the current NIH Guidelines.

Second of all, the use of the term "non-conjugative" is inappropriate because the actinomycetes do not exchange genetic information by the process of conjugation as it is known for eubacteria. Rather actinomycetes exchange genetic information by heterokaryosis. In some experiments, the frequency of genetic exchange can be altered by the presence of plasmids and some plasmids can mediate their own transfer to new hosts.

I feel that to restrict cloning in the actinomycetes to using "non-conjugative" or non-self-transmissible vectors would be unduly harsh and would severely restrict research with this valuable group of organisms. Considering the level of potential hazard (which I consider minimal) and the potential benefit (which I view as considerable), P2 containment is more than sufficient protection regardless of the vectors employed.

Sincerely,

Dean P. Taylor

Dean P. Taylor, Ph.D.
Associate Senior Investigator

DPT:jlb

Lilly Research Laboratories
A Division of Eli Lilly and Company
307 East McCarty Street
Indianapolis, Indiana 46285

I. S. Johnson, Ph.D.
Vice President

November 3, 1980

Dr. Donald S. Fredrickson
Director
Building One, Room 124
National Institutes of Health
Bethesda, Maryland 20014

Dear Dr. Fredrickson:

Re: Recombinant DNA Research

During the Research Advisory Committee meeting of September 25-26, several actions were taken which should facilitate the proper development of recombinant DNA technology. However, one recommendation adopted by the committee for your consideration is inconsistent with prior actions taken by the RAC and is in our view most inappropriate. Specifically, the RAC proposed the establishment of a permanent subcommittee of the RAC on large-scale practices. The responsibilities of that subcommittee are defined as follows:

> A large-scale review subcommittee of the RAC shall be established with the responsibility of advising the RAC on procedures and facilities designed pertaining to large-scale operations and on the performance of local IBC's in reviewing physical containment facilities. The subcommittee shall invite participation by the NIH Biosafety staff, NIOSH, OSHA, CDC, FDA, EPA, and USDA.

We consider the establishment of a subcommittee along the lines proposed to be ill-advised for the following reasons:

(1) The proposed subcommittee jurisdiction with respect to large-scale operations is inconsistent with prior RAC actions. The RAC has previously recognized that the increased volume involved in so-called "large-scale" experiments does not result in increased risks at least insofar as the escape of organisms is concerned. This is inherent in the RAC proposal to delete paragraph 2 of section I-D-6 of the Guidelines is noted in 45 F.R. 50526 (July 29, 1980).

Also, the recommendations made by Dr. Maxine Singer and Dr. Gottesman during the September RAC meeting, which were favorably considered, are indicative of the growing feeling of confidence expressed by responsible scientists in the day-to-day conduct of recombinant DNA research. The acceptance of these recommendations indicates a decreased desire for RAC oversight and greater reliance by RAC on IBC's.

(2) It does not appear desirable to create a new subcommittee with representatives from a half dozen regulatory agencies to complicate the review process applicable to large-scale protocols. The proposed activities of the subcommittee would certainly duplicate in part the functions of the Federal Interagency Advisory Committee on recombinant DNA research (which incidentally has an Industrial Practices Subcommittee). The agencies listed as participants in the proposed subcommittee are usually present at RAC meetings as observers. In addition, industrial applications of recombinant DNA technology are by statute subject to the laws these agencies administer, e.g., the Federal Food, Drug, and Cosmetic Act for pharmaceutical products, the Occupational Health and Safety Act for worker safety, and a number of other Federal acts administered by the EPA.

No evidence is presented which points toward the need for evaluation of the performance of IBC's which consider large-scale recombinant DNA applications. Evaluation, audit, performance standards, inspection, or other oversight procedures for IBC's should be considered only after a full discussion by the RAC of the need for such procedures for all types of recombinant DNA research activities. It is our understanding that the situation in California which may have prompted the RAC's concern did not involve a failure to act by the IBC but a failure to adhere to communication procedures by staff personnel.

For the reasons noted above, the creation of the subcommittee as proposed will result in duplication in existing oversight and advisory mechanisms; for example the RAC has already approved large-scale guidelines (Part VII) in addition to an existing working group on large-scale recombinant DNA activities. The creation of this subcommittee will focus inappropriately IBC activities solely relating to large-scale experiments and will not be responsive to the need for prompt consideration of large-scale submissions.

We therefore urge that such a subcommittee, if created, be granted authority similar to that of an "executive committee" which could act for and on behalf of the RAC with respect to large-scale submissions during those periods when the RAC is not in session. For the reasons stated above, such a subcommittee should not focus in a discriminatory manner on the activities of IBC's which may have considered or may consider large-scale recombinant DNA applications.

Your review and consideration of these comments is most appreciated. We would be glad to respond to any questions regarding these proposals.

Very truly yours,

Irving S. Johnson

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FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Minutes of the Eleventh Meeting
Wednesday, February 27, 1980
10:00 A.M. - 12:00 Noon

National Institutes of Health
Bethesda, Maryland 20205

Dr. Fredrickson noted first that at its meeting on July 17, 1979, the Committee reviewed an NIH proposal for extending the NIH Guidelines to private-sector research on a voluntary basis. There have since been a number of developments on recombinant DNA research, the Guidelines, and industrial applications of recombinant DNA technology. At today's meeting, the intent was to review those developments and to consider possible Committee actions in the industrial applications area. Next, Dr. Fredrickson turned to the agenda items. (See Attachment A for list of attendees.)

Recombinant DNA Research Developments

Recent Achievements Involving Recombinant DNA Technology. Dr. Fredrickson called on Dr. Maxine Singer, Acting Chief of the Laboratory of Biochemistry, Division of Cancer Biology and Diagnosis, National Cancer Institute, to review scientific advances. Dr. Singer noted that enormous progress has been made in hundreds of laboratories throughout the world, through recombinant DNA techniques coupled with techniques for determining the sequence of base pairs in DNA.

There have been two major areas of special scientific achievement: (1) isolation and purification of small fragments of DNA in order to study the structure and function of genes; and (2) manipulation of DNA to obtain genetic expression--i.e., the production of particular proteins.

Concerning the first area, Dr. Singer briefly explained the structure of genes and the role of messenger RNA in the production of protein, distinguishing the mechanism in bacteria from that of more complex organisms. In bacteria the messenger RNA is transcribed from the DNA and then translated to produce the protein. In more complex organisms, RNA is transcribed from the DNA but goes through another step to produce a mature messenger RNA before being translated to protein. This step involves excision from the RNA of transcripts of "intervening sequences." Some genes have 17 or more intervening sequences, each of which must be spliced out with extreme accuracy. The list of genes with intervening sequences is growing larger every day. Among them are the genes for interferon, prolactin, insulin, and growth hormone. Intervening sequences have enormous implications for understanding how genes of complex organisms work, for evolution, and for risk assessment.

In many recombinant DNA experiments, DNA segments are carried by vectors into E. coli. This organism lacks a mechanism for removal of intervening sequences and therefore cannot make the correct gene product if such sequences are present in the genes.

On the other hand it appears that a protein cannot be made in a eukaryotic host unless the inserted gene contains intervening sequences.

Another discovery is that there are changes in DNA structure during the process of differentiation. Dr. Singer gave examples in yeast and Salmonella, where certain characteristics are determined by DNA segments that move around. A striking case is the genes for immunoglobins, which are rearranged from fetal DNA to mature DNA.

Through recombinant DNA procedures, defects in globin production are being analyzed and diagnosis can now be made much more readily. Another major development is in the production of pharmaceutically important proteins, such as human growth hormone, insulin, and interferon. A lot of attention is being given to the problem of how to boost the level of protein synthesis in the E. coli used for such production.

Revision of the NIH Guidelines. Dr. William J. Gartland, Jr., Director of the NIH Office of Recombinant DNA Activities (ORDA), briefly reviewed the revisions of the Guidelines in the past year. He reported that the Recombinant DNA Advisory Committee (RAC), in September 1979, recommended exempting from the Guidelines most recombinant DNA experiments using the E. coli K-12 host-vector system.

In reviewing the RAC recommendations, Dr. Fredrickson decided that these experiments should not be exempt, but should be conducted at the P1-EK1 level, be registered with the local institutional biosafety committee (IBC), and require IBC approval for experiments in which a deliberate attempt is made to efficiently express a eukaryotic protein. This decision was published in November 1979 in the Federal Register for public comment, and promulgated on January 29, 1980. The Guidelines now have a new Section III-O, on containment for E. coli K-12 host-vector systems.

Dr. Fredrickson noted that the Guideline revisions have placed more responsibility on the local institutions. With these revisions, he sent a letter to the president or dean of all universities where such research is taking place, specifying the new responsibilities of the IBCs and enjoining strict containment at the P1 level.

Risk-Assessment Program. Dr. Richard Krause, Director of the National Institute of Allergy and Infectious Diseases, briefly reviewed the NIH risk-assessment program. He noted that most of the information relevant to risk-assessment is coming from basic science and not from specific risk-assessment studies. The latter have included looking at survival of organisms in such places as sewage or the GI tract of animals. The data have been presented to the RAC.

Two major topics will be considered at a risk-assessment meeting to be held in Pasadena in April. The first is the possibility of direct adverse effects of hormone-producing strains of *E. coli* K-12. The second is the possibility of autoantibodies or autoreactive cells due to the production of eukaryotic proteins by *E. coli* K-12.

Dr. Morris Levin of EPA then described his agency's risk-assessment effort. The mission of EPA is to protect and enhance the environment, and there is concern that the release of organisms containing recombinant DNA might be harmful. The Office of Research and Development at EPA is focusing on emerging environmental problems where anticipatory research is necessary, including applied genetics. Two workshops have been held. One, at the University of Rhode Island focused on sewage treatment. The issue is whether current processes for waste water treatment can deal with large-scale release of novel genomes. The other workshop, at the University of California, focused on the effect of novel genomes in the environment. EPA is soliciting grants and contracts in these areas. (See Attachment B for relevant document on EPA activities.)

Industrial Application of Recombinant DNA Technology

Recombinant DNA Guidelines--Voluntary Compliance (Part VI). Dr. Bernard Talbot, Special Assistant to the Director, NIH, summarized developments on voluntary compliance since the last interagency meeting. The supplement that the Committee had reviewed at its July 1979 meeting was published for public comment in the *Federal Register* in August and recommended by the RAC in September. It was published again in the *Federal Register* for comment on November 30 and incorporated in the Guidelines promulgated on January 29, 1980. It encourages voluntary compliance by industry, with protection for proprietary information. The RAC has considered requests from industry at its last two meetings. Large-scale requests were approved from Eli Lilly for insulin and from Genentech for insulin and somatostatin.

Proposed Guideline Supplement for Large-Scale Containment. Dr. W. Emmett Barkley, Director, Division of Safety, NIH, discussed a Part VII to the Guidelines which is being developed to describe physical containment for large-scale use of organisms containing recombinant DNA molecules. The RAC has considered a draft, which has been revised and will be resubmitted to the Committee at its March meeting. Three levels of containment are described: P1-LS, P2-LS, and P3-LS. Work at the P1-LS level must be confined to closed systems. At the P2-LS level, there are more requirements concerning the integrity of containment. At the P3-LS level, there are also specific design criteria for the facility in which the equipment is located.

Congressional Activities. Dr. Burke Zimmerman, Director, Division of Legislative Analysis, NIH, discussed S.2234, introduced by Senator Stevenson. This bill would require registration with HEW of recombinant DNA activities in the private sector. (See Attachment C for a copy of S.2234.)

OSHA Concerns About Industrial Applications of Recombinant DNA Technology . Dr. Fredrickson reported that Dr. Eula Bingham, Administrator of the Occupational Safety and Health Administration, had written to him expressing concern about potential hazards to workers exposed to recombinant

DNA. In response to her letter, he had suggested that these concerns be brought before the Committee. (See Attachment D for the exchange of correspondence.) Dr. Fredrickson then called on Dr. Bingham.

Dr. Bingham stated that the OSHA Act of 1970 empowered the agency to enforce standards to make the workplace safe, but there are specific gaps in the law. It covers the private sector; public employees and universities are generally not covered. OSHA relies on NIOSH to provide recommendations and criteria for standards. Given OSHA's mandate, a number of its constituents have urged the agency to act on occupational safety and health concerns in regard to recombinant DNA research. She observed that every day more industries are developing a capacity for the technology and that it is essential to consider if and when standards should be promulgated. The technology introduces a vast new area of unknowns in occupational safety and health. OSHA, said Dr. Bingham, cannot wait for several years to promulgate standards, but on the other hand, more information would be required before action could be taken. NIOSH and OSHA look to Dr. Fredrickson and this Committee for guidance and advice.

Dr. Anthony Robbins, Director, National Institute for Occupational Safety and Health, confirmed that his agency is also hearing from constituents who urge actions to provide recommendations for OSHA. There are problems of translation, however, in going from research guidelines to the regulatory mode. It is not clear that NIOSH will recommend standards. NIOSH is considering industrial hygiene studies in these industries. There is a lack of data on what exposures might be in the industrial setting.

Dr. Fredrickson said that in light of these concerns and the growing development of industrial application of recombinant DNA techniques, a subcommittee is needed to examine the issues. With the Committee's approval, a subcommittee was created with the following agencies represented: the Department of Agriculture, Department of Commerce, National Institute for Occupational Safety and Health of the Center for Disease Control, Food and Drug Administration, National Institutes of Health, Occupational Safety and Health Administration of the Department of Labor, Environmental Protection Agency, National Science Foundation, and the Office of Science and Technology Policy. The subcommittee is instructed to provide appropriate guidance to the full Committee on what steps, if any, need to be taken beyond the NIH Guidelines to cover occupational health in industrial production involving recombinant DNA technology. The subcommittee will be chaired by Dr. Gilbert Omenn of OSTP. The subcommittee should report at the next meeting of the full Committee on its progress, with a final report in the fall. (See Attachment E for a charge to the Subcommittee.)

Dr. Fredrickson thanked the Committee for its cooperation in the development of Federal recombinant DNA research policies. He added that the next meeting would be devoted largely to the work of the subcommittee. In the absence of further business, the meeting was adjourned at noon.

Respectfully submitted,

Joseph G. Perpich, M.D., J.D.
Associate Director for
Program Planning and Evaluation

ATTACHMENT A

LIST OF ATTENDEES
Meeting of February 27, 1980

Chairman

Dr. Donald S. Fredrickson
Director
National Institutes of Health

Executive Secretary

Dr. Joseph G. Perpich
Associate Director for
Program Planning and Evaluation
National Institutes of Health

DEPARTMENT OF AGRICULTURE

Dr. Clarence O. Grogan
Science and Education Administration
Cooperative Research

DEPARTMENT OF COMMERCE

Dr. George S. Gordon
Environmental Affairs Analyst

DEPARTMENT OF DEFENSE

Dr. William R. Beisel
Scientific Advisor
U.S. Army Medical Research Institute
of Infectious Diseases

DEPARTMENT OF ENERGY

Dr. Larry Devean
Geneticist
Health Effects Research Division
Office of Environmental Health
Research

ENVIRONMENTAL PROTECTION AGENCY

Dr. Morris Levin
Director of Innovative Research
Programs

Dr. Darryl Banks
Executive Assistant to the
Assistant Administrator
for Research and Development

EXECUTIVE OFFICE OF THE PRESIDENT

Dr. Gilbert S. Omenn
Assistant Director for Human
Resources and Social and
Economic Services
Office of Science and Technology
Policy

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

Office of the Assistant Secretary for Health

Dr. Lowell T. Harmison
Senior Advisor
Office of Health Policy, Research
and Statistics

Center for Disease Control

Dr. Anthony Robbins
Director
National Institute for Occupational Safety and Health, CDC

Dr. Kenneth Bridbord
Director
Office of Extramural Coordination
and Special Projects, NIOSH

Mr. Seth Pauker
Health Scientist
Office of Program Planning
and Evaluation, NIOSH

Food and Drug Administration

Dr. Timothy J. Henry
Staff Science Advisor

Dr. John C. Petricciani
Deputy Director
Division of Pathology
Bureau of Biologics

Dr. Michael Goldberg
Special Assistant to the
Director, FDA

National Institutes of Health

Office of the Director

Dr. Bernard Talbot
Special Assistant to the Director

Dr. Burke Zimmerman
Director
Division of Legislative Analysis

Mr. William T. Carrigan
Special Projects Officer
Office of Program Planning
and Evaluation

Mr. Peter Slavin
Division of Legislative Analysis

Ms. Florence Hassell
Division of Program Analysis

Office of the General Counsel

Mr. Richard J. Riseberg
Legal Advisor

National Cancer Institute

Dr. Maxine Singer
Acting Chief, Laboratory of
Biochemistry
Division of Cancer Biology
and Diagnosis

National Institute of Allergy and Infectious Diseases

Dr. Richard Krause
Director, NIAID

Dr. John E. Nutter
Chief, Office of Specialized
Research and Facilities

Dr. William Gartland
Chief, Office of Recombinant
DNA Activities

Dr. Stanley Barban
Office of Recombinant DNA
Activities

Dr. Elizabeth Milewski
Office of Recombinant DNA
Activities

National Institutes of Health (Cont.)

Office of Research Services

Dr. W. Emmett Barkley
Director
Division of Safety

DEPARTMENT OF LABOR

Dr. Eula Bingham
Administrator, Occupational
Safety and Health Administration

Dr. David Logan
Occupational Safety and Health
Administration

NATIONAL SCIENCE FOUNDATION

Dr. Herman W. Lewis
Head, Section on Cellular Biology
Division of Physiology, Cellular
and Molecular Biology

DEPARTMENT OF STATE

Mr. William J. Walsh III
Biomedical Research Liaison
and Health Affairs Officer
Bureau of Oceans and International
Environmental and Scientific
Affairs

VETERANS ADMINISTRATION

Mr. Howard M. Berman
Acting Chief
Program Review Division

1. Title of Project: Mini-Assessment Contracts for Selected Future Environmental Problems

2. Estimated Period of Performance: 6 Months

3. Contract Officer: Dr. Morris Levin
Office of Strategic Assessment &
Special Studies (OSASS)

4. Background

The Mini-assessment Program is one component of OSASS' overall 1980 program for meeting its assigned responsibilities. The small-scale studies undertaken as part of this program are intended to provide OSASS sufficient information on potentially signficant future environmental problems and analytical requirements in futures research to support resource allocation and research priority decisions. Each mini-assessment project focuses on a potentially significant future environmental problem and is intended to produce a report summarizing the state-of-knowledge concerning the problem, defining and scoping the problem in terms of its public health, public welfare and environmental policy implications and identifying major information/knowledge gaps and inadequacies in analytical methods and techniques.

As part of its FY 1980 program, OSASS wishes to have mini-assessments performed on eight topics, including:

1. Agricultural uses of applied genetics and biotechnologies; and

2. Industrial use of applied genetics and biotechnologies.

The attached bibliography and description of our concerns is intended to guide the potential contractors.

1. Applied Genetics: Industrial

Numerous articles have indicated that the Pharmaceutical Industry is increasing its investment in the area of Applied Genetics and Biotechnology. Most major firms are either expanding existing departments or creating new ones to enhance their competitive position. In addition, new corporations have been formed exclusively to exploit genetic engineering and a number of unrelated existing firms are entering the arena (Revlon, for example).

These facts indicate the potential magnitude of the effect of genetic engineering on the Pharmaceutical Industry. However, given the extensive research effort (300-400 million dollars in 1980) and the range of projects being undertaken, there is little doubt that other industries will be affected. For example, non-pharmaceutical, genetically oriented researchers are examining more efficient means to produce ethanol, methanol, and citric acid and the use of biological scavengers to degrade toxic wastes, pesticides and oil spills. Within the health-science industry attention is focusing on production of hormones (insulin, human growth hormone, interferon) and according to some reports the status is now equivalent to that of the antibiotic industry about 35 years ago.

There have been Congressional Hearings and the National Institute of Health has developed a set of guidelines but the thrust of these efforts have been to lower the degree of risk associated with small scale research efforts. The problems associated with commercial activity have not been explored. For example, would the governmental options for controlling a hazardous or potentially ecologically damaging situation be greatly affected if the product were a "miracle drug"?

This assessment should consider the potential ecologic, economic and social impact of the rapidly expanding applied genetics industry. The assessment must include a description of the potential of the problem (including likelihood, magnitude), as well as a comprehensive, integrated overview of the longer term regional, national, and global environmental outlook. This should permit the contractor to evaluate concepts, models, methods, and environmental controls and policies as they affect the environmental future.

The final report must summarize the state-of-knowledge concerning the problem, define and scope the problem in terms of its public health, public welfare and environmental policy implications and identify major information/knowledge gaps and inadequacies in analytical methods and techniques.

2. Applied Genetics: Agriculture

The major impact of applied genetic research over the next decade may well be in the realm of agriculture. The potential for expansion of Biotechnology via Applied Genetics into the industrial arena was discussed in the previous description of Applied Genetics: Industrial, and most of the same expansion factors mentioned there apply to agriculture as well. The addition of nitrogen fixing ability to non-leguminous plants, control of crop disease, production of fertilizer and degradation of pesticides are only a few of the potential agriculturally related products of genetic engineering. The positive result of these developments could be additional acreage available for food crops, lowered pesticide and fertilizer usage, or higher crop yields per acre resulting in increased food supply. The possible disbenefits include the potential for decreasing the gene pool in a given food stock, ecological or public health effects resulting from large scale dispersion of novel genomes, or the economic, ecologic, or social consequences of rapidly expanding the cultivation of a new species.

This assessment should consider the potential ecologic, economic and social impact of the rapidly expanding applied genetics industry. The assessment must include a description of the potential of the problem (including likelihood, magnitude), as well as a comprehensive, integrated overview of the longer term regional, national, and global environmental outlook. This should permit the contractor to evaluate concepts, models, methods, and environmental controls and policies as they affect the environmental future.

The final report must summarize the state-of-knowledge in terms of its public health, public welfare and environmental policy implications and identify major information/knowledge gaps and inadequacies in analytical methods and techniques.

1. "A "Wonder Drug" in the Making "; Business Week, No. 2612, Nov. 19, 1979; p. 71-4.

2. Crea, Roberto et al.; Chemical Synthesis of Genes for Human Insulin; Proceedings of the National Academy of Science; Sept. 24, 1979; p. 64.

3. "New Hope for Going to Market with DNA"; Business Week, No. 2604; Sept. 24, 1979; p..64.

4. Federal Register, part II; vol.41; July 7, 1976; p. 27902-43.

5. Federal Register, part III; vol.44; April 2, 1979; p.19301-04.

6. Federal Register, part IV; vol.45; January 29, 1980; p.6724-6749.

7. "Genetic Engineering: Benefits and Dangers". WHO Chronicle; Dec. 1978; p.465-468.

8. Genetic Engineering: Evolution of a Technological Issue; Supplemental Report I; 93rd Congress, 2d session; prepared by the Science Policy Research Service, Library of Congress; Washington, US Gov. Printing Office, December, 1974; p.215.

9. Health, Education and Welfare, Dept. of Public Health Service. National Institues of Health. The patenting of recombinant DNA research inventions developed under DHEW support: an analysis by the Director, National Institutes of Health. Washington, US Govt. Printing Office, Nov. 1977; p.18 plus appendix material.

10. "Recombinant DNA: Warming up for Big Payoff" Science; vol.206, 1979; p.663.

11. Recombinant DNA Bulletin, (NIH), vol. 5; p.2-8.

12. Recombinant Molecules - impact on science and society. Miles International symposium series, no.10; Raven Press, 1977; p.540.

13. Science, April 8, 1977; vol.196, no. 4286. Entire issue.

14. Seeburg, P.H. et al,; "Synthesis of Growth Hormone by Bacteria" Nature Dec. 21-28, 1978; p.795-798.

15. "Supreme Court to Say if Life is Patentable". Science, vol.664, no.4419; Nov. 9, 1979.

16. US Congress. House. Committee on Interstate and Foreign Commerce. Subcommittee on Health and the Environment. Recombinant DNA Research Act of 1977. Hearings 95th Congress, 1st session, on H.R. 4759, H.R. 4849.

H.R. 3191. H.R. 3591. H.R. 3592. HR.R 5020, H.R. 4232, and H. Res. 131. March 15-17, 1977. Washington, US Govt. Printing Office, 1977. p.480. "Serial no.95-18"

17. US. Congress House Committee on Interstate and Foreign Commerce. Recombinant DNA Act. Report (together with dissenting and separate views and including the Congressional Budget Office cost estimate) to accompany H.R. 11192. March 24, 1978. Washington, US Govt. Printing Office, 1978. p.44. (95th Congression, 2nd session. House. report no.95-1005, part I).

18. US Congress Housa. Committee on Science and Astronautics. Subcommittee on Science, Research and Technology. Genetic Engineering: evolution of a technology issue. 92nd Congress, 2nd session. Prepared by the Science Policy Research Division, Congressional Research Service, Library of Congress. Washington, US Govt. Printing Office, 1972. p.119.

19. US Congress. House. Committee on Science and Technology. Recombinant DNA Act. Report (together with dissenting views and including a Congressional Budget Office cost estimate) to accompany H.R. 11192. April 21, 1978. Washington, US Govt. Printing Office, 1978; p.32. (95th Congress, 2nd sessi House report no. 95-1005, part II).

20. US Congress House Committee on Science and Technology. Subcommittee on Science, Research, and Technology. Genetic engineering, human genetics, and cell biology: evolution of Technological issues - DNA recombinant molecule research; supplemental report II. 94th Congress, 2nd session. Prepared by the Science Policy Research Division, Congressional Research Service, Library of Congress. Washington, US Govt. Printing Office Dec. 1976; p.259. At head of title: 94th Congress, 2nd session committee print. (see appendixes for a selected bibliography, selected articles and Draft Environmental Impact Statement)

21. US EMBO workshop to assess risks for recombinant DNA experiments involving the genomes of animal, plant and insect viruses. Federal register, part II, March 31, 1978; p.13747-13755.

22. Wade, Nicholas; The Ultimate Experiment: Man-Made Evolution. Walker, 1977; p.162.

ATTACHMENT C

II

96TH CONGRESS
2D SESSION

S. 2234

To provide for notification to the Department of Health, Education, and Welfare by certain persons conducting recombinant DNA research, and for other purposes.

IN THE SENATE OF THE UNITED STATES

JANUARY 29 (legislative day, JANUARY 3), 1980

Mr. STEVENSON introduced the following bill; which was read twice and referred to the Committee on Labor and Human Resources

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A BILL

To provide for notification to the Department of Health, Education, and Welfare by certain persons conducting recombinant DNA research, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That this Act may be cited as the "Recombinant DNA Research and Development Notification Act of 1980".

FINDINGS

SEC. 2. The Congress finds that—

(1) research and other activities involving recombinant DNA increase our understanding of basic biological processes;

(2) the knowledge gained from such research and other activities may provide substantial benefits to medicine, industry, and agriculture;

(3) while no imminent hazards have been demonstrated, the extent to which organisms containing recombinant DNA present a risk of injury to the health of individuals and to the environment has not yet been determined; and

(4) until the uncertainties regarding the safety and potential benefits of recombinant DNA research and other activities are resolved, protection of the public health and welfare and the responsibilities of the Federal Government regarding the commerce of the United States are best served by requiring persons who conduct such research and activities to notify the Department of Health, Education, and Welfare of the conduct of such activities and the precautions taken to provide for the safe conduct of such activities.

DEFINITIONS

SEC. 3. For purposes of this Act, the term—

(1) "DNA" means deoxyribonucleic acid;

(2) "recombinant DNA" means either—

(A) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell; or

(B) DNA molecules that result from the replication of a molecule described in subparagraph (A) of this paragraph;

(3) "recombinant DNA activity" means the possession of recombinant DNA by any person, and any activity (including research and transportation) undertaken by any person for the production or use of recombinant DNA;

(4) "commencement of a recombinant DNA activity" means the date on which actual laboratory experimentation or growth media development is initiated, and such term shall not mean the date on which planning for such activity is initiated, unless such planning date coincides with the date of initiation of actual laboratory experimentation or growth media development; and

(5) "Secretary" means the Secretary of Health, Education, and Welfare.

NOTIFICATION

SEC. 4. (a)(1) Any person conducting recombinant DNA activity, other than a person conducting such activity at, or sponsored by, an institution that receives any support for recombinant DNA research from the National Institutes of

Health, shall submit a report of such activity and such additional information relating to such activity as the Secretary shall prescribe under paragraph (2) of this subsection to the Secretary. Such notification shall be transmitted to the Secretary within ten days after the date of enactment of this Act or within ten days after the date of commencement of a recombinant DNA activity which is undertaken on or after the date of enactment of this Act, and every twelve months thereafter until such activities are terminated.

(2) The Secretary shall prescribe the content and form of the information to be submitted to the Secretary under paragraph (1) of this subsection. Such information shall include, but need not be limited to—

(A) the individual's or entity's name;

(B) an identification of the place or places in which such research or activity is being conducted;

(C) an identification of the source of the DNA and the host-vector system employed;

(D) the level of physical containment of such activity;

(E) the volume of the growth media in which recombinant DNA organisms will be grown;

(F) an outline of the experiments to be conducted over the subsequent twelve-month period, including the stages of such experimentation, and the production goals of such activities; and

(G) the date on which such research or activity was commenced.

(b) Within ten days after receipt of such notification, the Secretary shall acknowledge such receipt in writing to such person.

PENALTIES

SEC. 5. (a) Any person required to notify the Secretary under section 4 of this Act who fails to notify the Secretary concerning the commencement or continuation of recombinant DNA research or activity in accordance with such section shall be liable to the United States for a civil penalty in an amount not to exceed $5,000 for each such violation. Each day such violation continues shall constitute a separate violation.

(b) Such a penalty shall be assessed by the Secretary by an order made on the record. Such order must be preceded by written notice to such person of the Secretary's intent to issue such order and an opportunity for a hearing pursuant to a request made within fifteen days after the date on which such notice is received, in accordance with section 554 of title 5, United States Code.

(c) Any person who has requested, in accordance with the provisions of subsection (b) of this section, a hearing respecting the assessment of a civil penalty and who is aggrieved by an order assessing a civil penalty may file a petition for judicial review of such order with the United States Court of Appeals for the District of Columbia Circuit or for any other circuit in which such person resides or transacts business. Such a petition may only be filed within the thirty-day period beginning on the date on which the order making such assessment was issued.

(d) If any person fails to pay an assessment of a civil penalty—

(1) after the order making the assessment has become a final order and if such person does not file a petition for judicial review of the order in accordance with the provisions of subsection (c) of this section; or

(2) after a court in an action brought under the provisions of subsection (c) of this section has entered a final judgment in favor of the Secretary;

the Attorney General shall recover the amount assessed (plus interest at currently prevailing rates from the date of the expiration of the thirty-day period referred to in subsection (c) of this section or the date of such final judgment, as the case may be) in an action brought in any appropriate district court of the United States. In any such action, the validity, amount, and appropriateness of such penalty shall not be subject to review.

ACCESS TO INFORMATION

SEC. 6. (a) The Secretary shall make available upon request any information which is submitted pursuant to this Act, except that information which is exempt from disclosure pursuant to section 552(b)(4) of title 5, United States Code, shall not be disclosed by the Secretary. In submitting information pursuant to this Act, a person may designate items of information considered by such person to be exempt from disclosure. Prior to making a determination pursuant to a request for release of such information under section 552 of title 5, United States Code, the Secretary shall consult with the person who submitted such information.

(b) If the Secretary determines that such information is not exempt from disclosure, the Secretary shall notify the person who submitted such information of such determination and that he intends to release such information. Such information shall not be released by the Secretary before the fifteenth calendar day following the date of such notification, unless the Secretary determines that release of such information is required to protect against an imminent hazard to the public or to the environment.

(c) Notwithstanding the provisions of this section, the Secretary may release any information upon a determination that such release is necessary to protect against an imminent hazard to the public or to the environment.

REPORT

SEC. 7. Not later than six months after the effective date of this Act, and annually thereafter, the Secretary, in consultation with the Secretary of Commerce, and the Director of the Office Science and Technology Policy, shall submit a report to the Committee on Labor and Human Resources and the Committee on Commerce, Science, and Transportation of the Senate, and the Committee on Interstate and Foreign Commerce and the Committee on Science and Technology of the House of Representatives, relating to the implementation of this Act in the prior twelve-month period. Such report shall include an evaluation of the notification requirement established by this Act, an assessment of the extent to which persons subject to this Act complied with applicable requirements, an assessment of the extent to which precautions reported to have been taken in the use of recombinant DNA were adequate to protect the health and welfare of individuals involved in research and other activities and the general public, and such other information or recommendations as the Secretary determines to be appropriate.

EFFECTIVE DATE

SEC. 8. The provisions of this Act shall take effect on the tenth day after the date of the enactment of this Act and shall terminate thirty-six months thereafter.

Eula Bingham, Ph.D.
Assistant Secretary for
Occupational Safety and Health
U.S. Department of Labor
Washington, D.C. 20210

Dear Eula:

In your letter of December 17, you raised issues concerning potential regulation of the industrial application of recombinant DNA technology that on earlier occasions we have discussed at least tentatively in the Federal Interagency Advisory Committee on Recombinant DNA Research. As you know, this Committee was created in October 1976 to serve as the Federal policy coordinator for recombinant DNA research and regulatory policies. Its membership (see attached) is broad and it is intended to provide an effective forum for dealing with the issues that concern any of the agencies having jurisdiction or other relevant interest in the use of recombinant DNA methodology.

I believe it is the most appropriate group to consider the points you raise. I will convene a meeting of the Committee within the month to consider them. One possible approach would be to create a special industrial production subcommittee to deal with the many issues that interest not only the Occupational Safety and Health Administration but also the Environment Protection Agency, and the Food and Drug Administration.

Sincerely yours,

/s/ Donald S. Fredrickson, M.D.

Donald S. Fredrickson, M.D.
Director

Enclosure

U.S. DEPARTMENT OF LABOR
Occupational Safety and Health Administration
WASHINGTON, D.C. 20210

Office of the Assistant Secretary

DEC 17 1978

Donald Fredrickson, M.D.
Director
National Institutes of Health
5600 Fishers Lane
Bethesda, Maryland 20014

Dear ~~Dr.~~ Don Fredrickson:

The Occupational Safety and Health Administration is concerned about potential health hazards to workers exposed to recombinant DNA. This concern is intensified by the increasing commercial uses of recombinant DNA in multiple diverse areas, the prospect of larger quantities being utilized in certain production processes, and the increasing number of individuals who will be exposed as the uses of recombinant DNA materials are further developed.

OSHA supports the program of voluntary compliance with the NIH recombinant DNA guidelines for research and use in areas outside its jurisdiction but feels that more formalized enforcement of procedures and rules within the regulated private sector is warranted. OSHA has a mandate to protect the health and safety of all workers, including workers involved in recombinant DNA research and utilization.

Because the nature of the hazards to individuals working with recombinant DNA remains somewhat theoretical, OSHA strongly urges that a major effort be undertaken to identify and describe the potential hazards to all exposed individuals. Only when the risks are better understood, can a strong enforcement program be undertaken. To help achieve this goal, we would like to recommend that an expert panel under the direction of David P. Rall be convened to specifically address the hazards of recombinant DNA use particularly as they apply to potential occupational exposures. Such a panel would certainly benefit from the active participation by members from the NIH Recombinant DNA Advisory Committee but the panel should also include technical representatives from unions, management, public interest groups, and other concerned parties. OSHA would be happy to help in the selection of the expert panel and would participate as needed.

OSHA would also support an active role by the National Institute for Occupational Safety and Health. A registry of all users of recombinant DNA material would be an important step in defining the scope and extent of use of recombinant DNA material and would facilitate continuing evaluation of any adverse health effects or toxicity. A survey of current users and industrial firms intending to use recombinant DNA and an evaluation of the experience with the current NIH guidelines could provide the basis for a joint NIOSH/OSHA health hazard alert or a Current Intelligence Bulletin.

OSHA could also review the NIH guidelines and publish them in an appropriate form as interim guidelines to industry. While these would not hold the force of regulations, they would indicate our support for control in this area and would also provide information to employers indicating what we feel are appropriate precautions and procedures of recombinant DNA use.

Finally, the Environmental Protection Agency should be encouraged to explore enforcement of recombinant DNA use through its jurisdiction derived from the Toxic Substance Control Act.

Initiation by OSHA of formalized standard setting is probably premature at the current time primarily because of limited understanding of the potential risks to workers involved with recombinant DNA and the absence of significant practical experience in industrial use. OSHA feels that the above mentioned steps by addressing the potential and real adverse health effects of recombinant DNA would help provide an important basis for future regulatory action.

We look forward to working with you and the Recombinant DNA Advisory Committee on this important public health concern.

Sincerely,

Eula

Eula Bingham
Assistant Secretary
Occupational Safety and Health

cc: Dr. David K. Parkinson
Dr. David Rall
Dr. Anthony Robbins

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : Gilbert Omenn, M.D., Ph.D.
Associate Director for Human Resources
and Social and Economic Services
Office of Science and Technology Policy

DATE: March 12, 1980

FROM : Director, NIH

SUBJECT: Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research

At its meeting on February 27, the Federal Interagency Advisory Committee on Recombinant DNA Research approved creation of an Industrial Practices Subcommittee. With the Committee's approval, I asked you to serve as Chairman; I am now writing to confirm this request.

I believe it important to review the considerations that led to the development of this Subcommittee and to its mandate. As reported at the meeting, several important scientific developments in recombinant DNA research over the past year involve industrial applications; among these are the production of insulin, growth hormone, and most recently interferon. These developments have directed new attention to the role of the industrial sector in recombinant DNA technology--an area in which NIH Guidelines apply only on a voluntary basis. It was against this background that Dr. Eula Bingham, Assistant Secretary for Occupational Safety and Health, Department of Labor, wrote to me in December 1979 expressing her concern about potential hazards to workers exposed to recombinant DNA technology. I suggested in response that this matter be brought before the Federal Interagency Advisory Committee; hence, our February 27 meeting.

The Committee discussed the issue of occupational health and Federal responsibilities attendant upon industrial applications of recombinant DNA technology. On the basis of that discussion it was agreed that a Subcommittee should be created to provide appropriate guidance for the full Committee on what steps need be taken, if any, beyond the NIH guidelines relating to industrial uses of recombinant DNA technology. It was felt that such a Subcommittee should meet at the earliest opportunity.

In considering issues of occupational health and other Federal responsibilities attendant upon industrial applications of recombinant DNA technology, the Subcommittee has the following responsibilities:

- To consider comments directed to member agencies concerning these issues;
- To provide a point of contact on these issues for persons outside the Federal Government and, where appropriate, to seek their advice, consultation, and participation in the work of the Subcommittee;

- To monitor and, where appropriate, to participate in the activities of the Recombinant DNA Advisory Committee and other activities of Federal agencies pertaining to these matters (such as the risk-assessment workshop to be held in April 1980 dealing with recombinant DNA and E. coli K-12, sponsored by the National Institute of Allergy and Infectious Diseases).

The following agencies shall be represented on the Subcommittee: Department of Agriculture; Department of Commerce; Department of Health, Education, and Welfare (National Institute for Occupational Safety and Health of the Center for Disease Control, Food and Drug Administration, National Institutes of Health); Department of Labor (Occupational Safety and Health Administration); Environmental Protection Agency; National Science Foundation; and the Office of Science and Technology Policy. Others may be added by the Subcommittee Chairman.

The issues to be addressed by your Subcomittee are of utmost importance to the scientific community, the public, and the Federal agencies with research and regulatory responsibilities in this area. Please keep me informed on the work of the Subcommittee. The Subcommittee should report on its progress at the next Interagency meeting, and prepare a final report for submission in the fall. The final report should contain recommendations on what actions, if any, the Interagency Committee should consider taking concerning occupational health and other Federal responsibilities attendant upon industrial applications of recombinant DNA technology. You have most important responsibilities, and I appreciate your willingness and that of the members of the Subcommittee to accept this demanding and difficult obligation.

Donald S. Fredrickson, M.D.

cc:
Members of the Industrial Practices Subcommittee

INDUSTRIAL PRACTICES SUBCOMMITTEE OF THE FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Minutes of First Meeting
Tuesday, April 1, 1980
2:00 to 3:30 p.m.
Room 3025, New Executive Office Building
Washington, D.C.

Chairman's Introduction

Dr. Omenn opened the meeting by reviewing Dr. Fredrickson's charge to the Subcommittee in his memorandum of March 12, and thanking Drs. Bingham and Robbins for having raised the important issue of occupational health attendant upon industrial applications of recombinant DNA technology.

History of Involvement of Industry With the NIH Guidelines

Dr. Talbot reviewed the involvement of industry with the NIH Guidelines, including:

o Promulgation in January 1980 of a new Part VI of the NIH Guidelines entitled "Voluntary Compliance" following endorsement of this Part in July 1979 by the Federal Interagency Advisory Committee on Recombinant DNA Research, and in September 1979 by the NIH Recombinant DNA Advisory Committee (RAC).

o Review by the RAC at each of its last three meetings, in closed session, of submissions from industry requesting to go over 10 liters in volume.

o A recommendation by the RAC at the last meeting that Physical Containment Recommendations For Large-Scale Uses of

Organisms Containing Recombinant DNA Molecules be published in the Federal Register. (Dr. Talbot reported that this will appear in the Federal Register next week and will be sent to all Subcommittee members.)

Status Report on OTA Study

Drs. Harsanyi and Kolsrud reported on the study by the Office of Technology Assessment, due to be completed in August, entitled "Impacts of Applied Genetics." Topics under study include: the state of the art of various genetic techniques (including recombinant DNA); the current and projected use by industry of these techniques; potential impacts on society, the economy, and the environment; distribution of funds in different areas; patentability of life forms; potential hazards to workers; and costs and benefits of the new techniques.

The ultimate aim of the study is to identify issues that may be of interest to Congress and to present a series of options. Dr. Harsanyi spoke of two conflicting goals--either promoting the industry or restricting activities in the area, e.g., to insure safety. The report will look at what Congress could do to further each of these.

Dr. Omenn said he disagreed that there is necessarily a dichotomy between safety and industrial application. He said an objective of this Subcommittee is to promote both. Dr. Harsanyi replied that many companies say regulation of any kind slows their efforts.

Presentation by OSHA and NIOSH

Dr. Bingham said that by law the Occupational Safety and Health Administration must respond to workers' complaints of an unsafe workplace, whether it involves a faulty crane or a biohazard. OSHA looks to NIOSH for recommendations as to whether or not a standard is necessary. There is a level of concern about recombinant DNA, and it would be remiss of the Federal Government to allow hysteria to develop among workers when this can be headed off by responsible Government action.

Dr. Robbins said that NIOSH has not yet had time to firmly establish its position with regard to occupational health in industrial uses of recombinant DNA. When looking at a new area, in deciding whether to recommend to OSHA that they act, NIOSH takes into account:

- data on toxicity
- the potential number of exposed workers
- the potential level of exposure to the worker
- the likelihood of a standard achieving a beneficial effect

NIOSH has a right of entry and, by visits to industry, can examine industrial hygiene and how exposures might occur. Based on submissions industry has already made to NIH, NIOSH can look at the current technology for minimizing exposure to workers--"control technology assessment." NIOSH would like to consider developing ways to increase worker understanding of recombinant DNA technology. In the case of recombinant DNA, the newness of the industry means that if recommendations can be brought forward quickly, they can be adopted in the "initial capitalization" of the industry, without the problem of "retrofit."

Drs. Bingham and Robbins both said they thought it inappropriate for the same government unit to be involved in both promoting a technology and protecting against its possible hazards.

General Discussion

Dr. Omenn endorsed the systematic review of potential risks and cited as a step in this direction the upcoming Recombinant DNA Risk Assessment Workshop, to be held in Pasadena, California, April 11-12. Many of the Subcommittee members indicated they would be attending.

Dr. Barkley said that, in his view, the NIH Guidelines for Recombinant DNA Research had done much to educate scientists in good laboratory practices in general. Even though he believes that recombinant DNA itself is not a major industrial microbiological hazard, the building of a consensus on appropriate control technology in this area could have important implications for controlling more serious hazards.

Dr. Robbins said that a registry of workers in this field should be begun now on a voluntary basis. Dr. Omenn said he hoped that when NIOSH has a more detailed plan for such a registry, it would be discussed with this Subcommittee.

Dr. Goldberg spoke of an inspection referral system, instituted by the Interagency Regulatory Liaison Group, whereby inspectors from one agency, noting a possible violation in the regulatory domain of another agency, make the appropriate referral.

Dr. Hunt described EPA's regulatory authorities and Dr. Banks noted that EPA has recently issued a solicitation for grant proposals, including studies of the potential impact of new genomes introduced into established ecosystems.

Dr. McCracken spoke of the great promise of recombinant DNA technology for agriculture.

Next Subcommittee Meeting

Dr. Omenn reminded the Subcommittee of Dr. Fredrickson's request for a final report by the autumn. Dr. Robbins said that NIOSH and OSHA staff would be contacting Subcommittee members and other staff of the agencies, over the next months, to ask for further information and for review of drafts. He requested a wait of about two months before the next Subcommittee meeting so that NIOSH could visit more industrial facilities and prepare its recommendations for presentation to the Subcommittee. Dr. Goldberg noted that FDA will be sponsoring a State of the Art Conference on Insulins and Growth Hormone, to be held at NIH on June 3 and 4. It was agreed to schedule the next Subcommittee meeting for after that date. Dr. Omenn asked if the Subcommittee should sponsor a public meeting. It was agreed that rather than have a large, open, public meeting, the next Subcommittee meeting, in June, would consist of 1 to 1½ hours of hearing from no more than six invited representatives of labor, industry, and/or science, to be immediately followed by another 1½ hour meeting of just the

Subcommittee. Subcommittee members were asked to suggest speakers for the June meeting and to telephone their suggestions to the Subcommittee executive secretary.

Bernard TALBOT

Bernard Talbot, M.D., Ph.D.
Subcommittee Executive Secretary
April 7, 1980

LIST OF ATTENDEES

Meeting of April 1, 1980

CHAIRMAN

Dr. Gilbert S. Omenn
Associate Director for Human
Resources, Veterans, and Labor
Office of Management and Budget

EXECUTIVE SECRETARY

Dr. Bernard Talbot
Special Assistant to the Director, NIH

DEPARTMENT OF AGRICULTURE

Dr. Ralph J. McCracken
Associate Director
Science and Education

DEPARTMENT OF COMMERCE

Dr. George Gordon
Office of Environmental Affairs

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

OFFICE OF THE ASSISTANT SECRETARY FOR HEALTH

Dr. Lowell T. Harmison
Science Advisor
Office of Health Policy, Research,
and Statistics

CENTER FOR DISEASE CONTROL

Dr. John H. Richardson
Director, Office of Biosafety

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH, CDC

Dr. Anthony Robbins
Director, NIOSH

Mr. Seth Pauker
Health Scientist
Office of Program Planning
and Evaluation

Mr. Larry Elliott
Industrial Hygienist
Division of Surveillance,
Hazard Evaluation, and
Field Studies

Mr. Denny Dobbin
Chief, Policy Analysis Branch
Office of Program Planning
and Evaluation

NATIONAL INSTITUTES OF HEALTH

Dr. W. Emmett Barkley
Director, Division of Safety

FOOD & DRUG ADMINISTRATION

Dr. Michael Goldberg
Special Assistant to the
Director, FDA

Dr. John C. Petricciani
Deputy Director, Division of
Pathology
Bureau of Biologics

Dr. Timothy J. Henry
Staff Science Advisor

Dr. Henry Miller
Medical Officer
Bureau of Drugs

DEPARTMENT OF LABOR

Dr. Eula Bingham
Assistant Secretary for
Occupational Safety and Health

Dr. David Logan
Medical Officer, Directorate
of Technology Support

Attendees continued -

ENVIRONMENTAL PROTECTION AGENCY

Dr. Vilma R. Hunt
Deputy Assistant Administrator
for Health Research

Dr. Darryl Bank
Executive Assistant to the Assistant
Administrator for Research and
Development

NATIONAL SCIENCE FOUNDATION

Dr. Herman Lewis
Head, Section on Cellular Biology
Division of Physiology, Cellular
and Molecular Biology

EXECUTIVE OFFICE OF THE PRESIDENT

Dr. Robert Harris
Member, Council on Environmental Quality

OFFICE OF TECHNOLOGY ASSESSMENT

Dr. Zsolt Harsanyi
Project Director, Genetics

Dr. Gretchen Kolsrud
Group Manager, Genetics and Population

INDUSTRIAL PRACTICES SUBCOMMITTEE
OF THE FEDERAL INTERAGENCY
ADVISORY COMMITTEE ON RECOMBINANT
DNA RESEARCH

Minutes of Second Meeting*
Wednesday, June 18, 1980
1:00 to 4:00 p.m.

Room 3104, New Executive Office Building
Washington, D.C.

PART I - MEETING WITH INVITED REPRESENTATIVES

Dr. Omenn opened the meeting by welcoming the invited representatives. Dr. Robbins said that he had previously requested that copies of his memorandum of June 11 to Dr. Omenn be sent only to federal officials and not to the invited representatives, so that the latter would focus at this meeting on their own views and concerns, rather than reacting to the NIOSH memorandum. Now, however, he had no objection to the memorandum being distributed to anyone requesting it. (The memorandum is appended as Attachment C.)

Ms. Margaret Seminario

Dr. Omenn called on Ms. Margaret Seminario, Industrial Hygienist, AFL-CIO, representing Mr. George Taylor, Director of the Division of Occupational Safety and Health, AFL-CIO. She indicated that a formal statement by the AFL-CIO will be submitted soon. (Executive Secretary's note - Since the statement was not received by the time these minutes were completed, it is not appended now, but will be forwarded separately to the Subcommittee when received.) Ms. Seminario indicated that AFL-CIO concerns with regard to the safety of those working with recombinant DNA have been transmitted over the past five years to OSHA, NIOSH, and NIH. It is the view of the AFL-CIO that OSHA is the agency with the mandate to protect the health of workers. The AFL-CIO looks to OSHA and NIOSH to develop regulatory programs. There is perhaps not enough information for OSHA to draft regulations for the recombinant DNA industry today, but steps should be taken to get there. NIOSH and OSHA should jointly make visits to plants to evaluate the industry and come up with criteria to protect workers. These might include environmental monitoring, medical surveillance, and perhaps most important, training and education. The AFL-CIO is willing to work with OSHA, NIOSH, NIH, and any other agency, to move toward measures to protect workers.

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* The planned agenda for the meeting as distributed to the Subcommittee on June 11 is appended as Attachment A, and the list of attendees as Attachment B. Part I of the meeting lasted until 3:00 p.m., and Part II until 4:00 p.m. A list of members of the Subcommittee is appended as Attachment K.

Dr. John Adams and Dr. Ann Skalka

Dr. Omenn next called on Dr. John Adams, Vice-President of the Pharmaceutical Manufacturers Association. He was accompanied by Dr. Ann Skalka of the Roche Institute of Molecular Biology, who is chairperson of the Working Group on Risk Assessment of the Committee on Genetic Experimentation (COGENE) of the International Council of Scientific Unions. (Dr. Adams' prepared statement and the first and second reports of the COGENE Working Group are appended as Attachments D, E and F).

Dr. Adams said that there is nothing novel or unique in the routine procedures used in recombinant DNA research and production that would suggest the need for extraordinary precautions. The safety record of industry in production of substances by fermentation has been outstanding. By comparison to other industrial processes, including the production of potent chemicals and biological products, the risk to workers and to the public of industrial recombinant DNA technology is probably minimal.

Following Dr. Adams' statement, Dr. Barkley asked whether perhaps new, small, industrial concerns which have not had the experience of PMA member firms, should be an area of special concern for the Subcommittee. Dr. Adams replied that many of these firms have great scientific competence and that they themselves often do not perform large-scale production. Dr. Robbins asked how workers are informed of hazards and of means to deal with them. Dr. Adams said this varied from firm to firm, but generally speaking, personnel are very well advised on risks and precautions. Dr. Omenn said it would be helpful to this Subcommittee to receive detailed information on how training and education concerning hazards are conducted. Dr. Adams said he will provide such information.

Dr. Christine Oliver

Dr. Omenn next called on Dr. Christine Oliver, Occupational Physician with the Oil, Chemical and Atomic Workers International Union, representing Mr. Anthony Mazzocchi, Director of Health and Safety for the Union. (Dr. Oliver's prepared statement is appended as Attachment G.) She called for regulations, with the force of law behind them, and with appropriate penalties for violations, to govern industrial use of recombinant DNA technology. These regulations should address: workers' right-to-know; environmental monitoring; biological monitoring and medical surveillance; emergency spills; and worker education.

Several sections of Dr. Oliver's statement elicited comment. When she said, "The expanded use of recombinant DNA technology introduces the possibility of genetic manipulation of this 'hypersusceptible' worker to modify him or her to make him/her compatible with the work environment," there were a number of questions from the Subcommittee, and a comment from Dr. Miller that this demonstrated an unfortunate tendency toward mystical thinking about recombinant DNA. Dr. Oliver replied that she did not believe such "human genetic engineering" was going on today, but that it is a future possibility one should be worried about.

She was also questioned when she said, "The use of this technology for commercial production, particularly in the chemical and mining industries, may well result in significant job loss . . . . The economic and health effects will be quite serious." She indicated that she meant that job loss could adversely affect health and that it is better to reduce exposure than to lose jobs. Others pointed out that significant hazards to workers today using conventional technology might be reduced in future processes employing recombinant DNA techniques.

When she said, "The Recombinant DNA Advisory Committee (RAC) has proposed (Federal Register, April 30, 1980, pp. 28904ff) the amending of the Guidelines to require membership by industry representatives," this was corrected by Dr. Johnson who noted that as stated in the Federal Register, the proposal was not the RAC's but rather his, and that it had been rejected by the RAC.

Dr. Miller wondered whether Dr. Oliver's and the Subcommittee's priorities should not be directed toward known rather than highly theoretical risks. Dr. Oliver replied that she believed there was not enough information to say that recombinant DNA presents no significant health hazard.

Dr. Irving Johnson and Dr. Bernard Davis

Dr. Omenn next called on Dr. Irving Johnson, Vice-President of Research, Lilly Research Laboratories. He was accompanied by Dr. Bernard Davis, Professor of Bacterial Physiology, Harvard Medical School. Dr. Johnson distributed to the Subcommittee a lengthy statement plus three attachments giving: features of fermentors; a paper entitled "L-Asparaginase - A Case Study of an *E. coli* Fermentation Product"; and report on "Site Visit, Eli Lilly Co., Large-Scale Recombinant DNA Production Facility." (These are appended as Attachment H.)

Dr. Johnson discussed five principal items: the origin of the 10-liter limitation; the safety of E. coli K-12 as determined by recent risk assessment programs; the reliability and operating procedures of commercial scale fermentation equipment, including training of operators; medical surveillance program for equipment operators; and the current authority of OSHA and the FDA. He also said he agreed with Dr. Bingham's statements in her letter of December 17, 1979, to Dr. Fredrickson (appended as Attachment I) that "only when the risks are better understood, can a strong enforcement program be undertaken" and that "addressing the potential and real adverse health effects of recombinant DNA would provide an important basis for future regulatory action." He said at present there are no known real adverse health effects of recombinant DNA.

Dr. Bernard Davis said E. coli K-12 is like a "hot house plant" which will not survive in the wild in competition with "weeds." In reply to those who say recombinant DNA could be more dangerous than chemicals because organisms can multiply, he noted that chemicals are stable while organisms either multiply or die out, and the ability for self-destruction can be built into them. He said that there has been hysteria about recombinant DNA, and that there should be positive evidence of risk before expensive regulatory schemes are put in place. There is no biological reason to expect any recombinant to be more dangerous than the host or the donor of the inserted DNA. Worker health is not protected by diverting time and effort away from real hazards to hypothetical hazards.

Dr. Robbins said that one must look beyond the organism to the products to which workers are exposed. There is an unfortunate tradition of industry not telling workers about the hazards to which they are exposed. Perhaps the good practices of some companies can be detailed and put forward as minimal standards for protecting workers. Dr. Johnson urged that any standards be written in terms of ends rather than means, so that companies can use engineering creativity in meeting the objectives. He also said that during a period of uncertainty and risk assessment, Lilly and others had prudently taken extensive steps, which are probably in excess of those required based on today's knowledge. He urged that these not be considered "minimal standards."

Mr. Mark Chatigny

Dr. Omenn next called on Mr. Mark Chatigny, Assistant Director, Naval Biosciences Laboratory, School of Public Health, University of California, Berkeley. He reviewed his extensive experience in dealing with contamination control measures and safety program operating procedures. He said the effectiveness of physical containment has been demonstrated in work with known pathogens. The NIH Recombinant DNA Guidelines not only dictate physical containment, but also biological containment. Compliance with the NIH Guidelines is widespread. The Guidelines provide the internationally accepted state of the art and appear to adequately protect workers for the recombinant DNA systems currently in use. Large-scale growth of recombinant DNA organisms uses only fully characterized clones in physically well-contained fermentors. Indeed, the growth of such organisms seems much less hazardous than the subsequent concentration and purification of such compounds as human growth hormone.

In concluding the first part of the meeting, Dr. Omenn thanked the invited representatives and indicated that they would be placed on a mailing list to receive these and all future minutes of meetings of this Subcommittee.

PART II - MEETING OF THE SUBCOMMITTEE ALONE

Report from NIH

Dr. Talbot reported that at the June 5-6 meeting of the NIH Recombinant DNA Advisory Committee (RAC), the item which took the greatest amount of time--many hours over the two days of the meeting--was a consideration of modification of procedures for reviewing requests to exceed 10-liters in volume. These are primarily requests voluntarily submitted by industry.

Dr. Fredrickson, the NIH Director, spoke to the Committee at the beginning of the meeting. He said he was sympathetic to the unease of many RAC members about their role. He felt the RAC should continue to look at biological containment in such proposals but might consider no longer reviewing the details of physical containment. (Dr. Fredrickson asked that copies of the introductory remarks he made at the RAC meeting be given to this Subcommittee. They were distributed, and are appended as Attachment J). After hours of deliberation on this issue, the RAC voted 17 to 0, with one abstention, to publish the following statement in the Federal Register, for public comment, to be reconsidered by the RAC at its next (September 25-26) meeting: "For approval of requests to grow greater than 10-liters of organisms containing recombinant DNA: RAC will determine if a given recombinant DNA-containing strain is 'rigorously characterized and the absence of harmful sequences established.' Such a determination shall include specification of a containment level (P-LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industrial scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC."

The effect of this would be to end consideration by the RAC on such requests, of details of laboratory procedures, fermentor design, etc. The RAC would continue to review the biology of the systems and to set an appropriate physical containment level, but would not review whether a given facility meets that level. This proposal will be published in the Federal Register at least 30 days prior to the September RAC meeting, along with details on how NIH proposes to implement it. When the material is sent to the Federal Register in August, copies will be sent to each Subcommittee member. Comments in response to the Federal Register notice will be welcome and will be considered by the RAC and the NIH. During the discussion of this issue at the RAC meeting, it was pointed out that final adoption of this change could leave a void in the review of such industry proposals. It was requested that this Interagency Subcommittee and its federal regulatory agency members be apprised of these possible changes. Some RAC members who felt strongly that regulatory agencies should step into this void proposed various motions to that effect. Other RAC members, however, felt that recombinant DNA should not, in their view, be a high priority of OSHA, compared with other known hazards, and all such motions concerning RAC advice to regulatory agencies were defeated.

Following Dr. Talbot's summation, Mr. Pauker said that the RAC had stressed the importance of medical surveillance in any regulatory process. Drs. Lewis and Talbot, however, noted that a motion to this effect had specifically been voted down at the RAC meeting.

Dr. Logan said that the apparent belief of some RAC members that OSHA and NIOSH can certify proposals before they are instituted is a misconception. He thought the RAC's proposed change in procedures for review of large-scale proposals should perhaps be discussed by this Subcommittee, which could consider making a formal recommendation to the RAC. Dr. Lewis said he felt that most of the RAC members did not have a misconception of OSHA's authorities. He noted that the motion passed by the RAC specifically names the Institutional Biosafety Committee as responsible for monitoring adherence to the specified containment level, and that words dealing with regulatory processes and regulatory agencies were specifically deleted from the motion before passage by the RAC. Dr. Omenn noted that the RAC has a tradition of relying on IBCs for monitoring, and said he felt that this Subcommittee need not deal with the issue at this time.

Report from NIOSH

Dr. Robbins discussed his June 11 memorandum to Dr. Omenn (appended as Attachment C). He said that NIOSH will continue looking at the eight items cited in the memorandum, i.e., physical containment design (e.g., fermentors); engineering controls (e.g., ventilation, filter of exhaust gases); work practices (e.g., operational protocols, clothing and hygiene requirements); validation procedures (e.g., sterilization and fermentor containment); emergency procedures (e.g., spills); environmental monitoring; medical surveillance (e.g., serum samples for serologic changes, monitor possible physiologic changes related to product exposure); and worker education. At the next Subcommittee meeting, a progress report will be presented. In developing that, NIOSH may be calling on many of the Subcommittee members for assistance. Dr. Robbins said that it is clear that the OSHA Act already covers almost all workers except some employees of state governments and state universities. Often, it is helpful to identify the best practices currently used in an industry and to make them broadly available so that all can follow them. Sometimes, some of these practices are so clearly better or so important that they become an OSHA standard.

Dr. Robbins said that much openness in the scientific community is disappearing. NIOSH is being described as a regulatory agency, and doors are closing. As an example, NIOSH wrote to NIH requesting copies of submissions made to NIH by private companies for large-scale recombinant DNA projects (see page 4 of Attachment C). Following the procedures specified in the Guidelines, NIH informed the companies of NIH's intent to release the information to NIOSH. The companies objected and said they would prefer that NIOSH request the material directly from them. NIOSH agreed to do so. Dr. Robbins said, however, that a principle of information exchange among government agencies was involved and he might want to come back to this issue.

Report from FDA

Dr. Goldberg noted that while OSHA is primarily concerned with process, FDA and USDA are primarily concerned with product. Dr. Miller described the June 3-4 FDA-sponsored "State Of The Art Conference on Insulins and Growth Hormone." It was reported there by industry representatives that insulin, growth hormone, and interferon made by recombinant DNA techniques are showing virtually identical biological activity to these products made by conventional techniques. This is true even though the growth hormone as currently made by recombinant DNA techniques has an extra methionine residue compared to the native human hormone, and even though the interferon made by recombinant DNA techniques is not glycosylated. No toxicity of the recombinant DNA-made products was demonstrated in animal tests including those in primates. Recombinant DNA-derived interferon was extremely bioactive in protecting against a lethal dose of virus in squirrel monkeys. FDA will soon be receiving applications to test recombinant DNA-made pharmaceuticals in man. Such products will require batch-to-batch analysis for identity by "fingerprinting" techniques since there is the possibility of coding sequence mutationsin a cloned gene. Possible toxicity, for example from unusual immunogenicity, will be looked for closely in preclinical and clinical studies. Recombinant DNA-made pharmaceuticals will require all the usual FDA checks of safety and efficacy. The only difference from conventionally derived drugs will be that a company with an already existing IND or NDA for a drug prepared by conventional means will not be allowed to substitute a recombinant DNA-derived product under an existing application, but rather will be required to file a new IND and NDA.

Plans For The Next Meeting

Dr. Omenn announced that the next Subcommittee meeting would be on Thursday, September 4, from 1:00 to 3:00 p.m. in Room 3104, New Executive Office Building.

Dr. Harmison said there is more to consider about potential hazards than worker safety, and he hoped EPA could report from their point of view considering potential hazards to the general population. Dr. Levin agreed to make a presentation at the next Subcommittee meeting concerning EPA's activities, including their solicitation of grant proposals in the area of applied genetics and of contract proposals for Mini-Assessment of Potentially Significant Future Environmental Problems, including "Applied Genetics: Industrial" and "Applied Genetics: Agriculture."

Dr. Omenn placed the following four items on the agenda for the September 4 Subcommittee meeting:

1. NIOSH should present illustrative material on worker education about biotechnologies, as assembled from its site visits and from material the Pharmaceutical Manufacturers Association will supply.

2. Illustrative examples should be presented by Subcommittee members including FDA, DOC, USDA and NIOSH as to the hazard of making the same product by recombinant DNA as compared to other methods. Dr. Goldberg's distinction of product versus process is important. The Subcommittee must look beyond recombinant DNA to the broader context of biohazards to convince itself that the priority in focusing on recombinant DNA is correct.

3. NIOSH should have further information on the progress it has made in evaluating the eight items cited in Dr. Robbins' June 11 memorandum.

4. EPA should report on its activities.

Additional agenda items may be suggested by Subcommittee members to Dr. Talbot.

Bernard Talbot, M.D., Ph.D.
Subcommittee Executive Secretary
June 20, 1980

SECOND MEETING OF THE INDUSTRIAL PRACTICES SUBCOMMITTEE OF THE FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Wednesday, June 18, 1980
1:00 to 3:00 p.m.
Room 3104, New Executive Office Building
Washington, D.C.

AGENDA

art I - Meeting With Invited Representatives

1:00 to 1:15 p.m. -

Mr. George H. R. Taylor
Director
Department of Occupational Safety and Health
AFL-CIO

Ms. Margaret Seminario
Industrial Hygienist
AFL-CIO

1:15 to 1:30 p.m. -

Dr. John Adams
Vice-President
Scientific & Professional Relations
Pharmaceutical Manufacturers Assn.

Dr. Ann Skalka
Department of Cell Biology
Roche Institute of Molecular Biology

1:30 to 1:45 p.m. -

Dr. Christine Oliver
Occupational Physician
Oil, Chemical and Atomic Workers International Union

1:45 to 2:00 p.m.

Dr. Irving S. Johnson
Vice-President of Research
Lilly Research Laboratories
Eli Lilly and Company

Dr. Bernard Davis
Professor of Bacterial Physiology
Harvard Medical School

2:00 to 2:15 p.m.

Mr. Mark A. Chatigny
Assistant Director
Naval Biosciences Laboratory
School of Public Health
University of California, Berkeley

Part II - Meeting of the Subcommittee Alone

2:15 to 2:20 p.m. - Dr. Bernard Talbot, Report from NIH

2:20 to 2:30 p.m. - Dr. Anthony Robbins, Report from NIOSH

2:30 to 2:35 p.m. - Dr. Michael Goldberg, Report from FDA

2:35 to 2:55 p.m. - Committee Discussion

2:55 to 3:00 p.m. - Dr. Gilbert Omenn
Conclusion and Plans for Future
Subcommittee Meetings

LIST OF ATTENDEES
Meeting of June 18, 1980

Page 1 of Attachment B

CHAIRMAN

Dr. Gilbert S. Omenn
Associate Director for Human Resources, Veterans, and Labor
Office of Management and Budget

EXECUTIVE SECRETARY

Dr. Bernard Talbot
Special Assistant to the Director, NIH

DEPARTMENT OF AGRICULTURE

Dr. Ralph J. McCracken
Associate Director
Science and Education

Dr. Sue Tolin
Science and Education Administration

DEPARTMENT OF COMMERCE

Dr. George Gordon
Office of Environmental Affairs

DEPARTMENT OF HEALTH AND HUMAN SERVICES

OFFICE OF THE ASSISTANT SECRETARY FOR HEALTH

Dr. Lowell T. Harmison
Science Advisor, Office of Environmental Affairs

CENTER FOR DISEASE CONTROL

Dr. John H. Richardson
Director, Office of Biosafety

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH, CDC

Dr. Anthony Robbins
Director, NIOSH

Mr. Seth Pauker
Health Scientist, Office of Program Planning and Evaluation

Mr. Larry Elliott
Industrial Hygienist
Division of Surveillance, Hazard Evaluation, and Field Studies

Mr. Melvin Myers
Director, Office of Program Planning and Evaluation

NATIONAL INSTITUTES OF HEALTH

Dr. W. Emmett Barkley
Director, Division of Safety

FOOD & DRUG ADMINISTRATION

Dr. Michael Goldberg
Special Assistant to the Director, FDA

Dr. John C. Petricciani
Deputy Director, Division of Pathology
Bureau of Biologics

Dr. Timothy J. Henry
Staff Science Advisor

Dr. Henry Miller
Medical Officer
Bureau of Drugs

DEPARTMENT OF LABOR

Dr. David Logan
Medical Officer, Directorate of Technology Support, OSHA

Dr. Lucile Adamson
Consultant, Division of Health Standards, OSHA

Dr. Chia T. Chen
Senior Industrial Hygienist
Office of Technical Support, OSHA

ENVIRONMENTAL PROTECTION AGENCY

Dr. Morris Levin
Director, Innovative Research Program

NATIONAL SCIENCE FOUNDATION

Dr. Herman Lewis
Head, Section on Cellular Biology
Division of Physiology, Cellular and Molecular Biology

EXECUTIVE OFFICE OF THE PRESIDENT

COUNCIL ON ENVIRONMENTAL QUALITY

Dr. Robert Harris
Member, Council on Environmental Quality

OFFICE OF SCIENCE AND TECHNOLOGY POLICY

Dr. Richard Adamson
Senior Policy Analyst

OFFICE OF TECHNOLOGY ASSESSMENT

Dr. Zsolt Harsanyi
Project Director, Genetics

Dr. Gretchen Kolsrud
Group Manager, Genetics and Population

INVITED REPRESENTATIVES (ATTENDED ONLY PART I OF MEETING)

Ms. Margaret Seminario
Industrial Hygienist
AFL-CIO

Dr. John Adams
Vice-President
Scientific & Professional Relations
Pharmaceutical Manufacturers Association

Dr. Ann Skalka
Department of Cell Biology
Roche Institute of Molecular Biology

Dr. Christine Oliver
Occupational Physician
Oil, Chemical and Atomic Workers International Union

Dr. Irving S. Johnson
Vice-President of Research
Lilly Research Laboratories
Eli Lilly and Company

Dr. Bernard Davis
Professor of Bacterial Physiology
Harvard Medical School

Mr. Mark A. Chatigny
Assistant Director
Naval Biosciences Laboratory
School of Public Health
University of California, Berkeley

Page 1 of Attachment C

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

DATE: JUN 11 1980

TO : Dr. Gilbert S. Omenn
Chairman, Industrial Practices Sub-Committee
of the Federal Interagency Advisory Committee
on Recombinant DNA Research

FROM : Anthony Robbins, M.D.
Director, NIOSH

SUBJECT: NIOSH's Proposed Program for Addressing Potential Hazards to Workers Related to Commercial Recombinant DNA Applications

This memorandum outlines NIOSH's program for addressing potential hazards to workers related to commercial recombinant DNA applications. It is important to consider how NIOSH concerns fit into the concerns of other representatives on this committee and of the Recombinant DNA Advisory Committee for potential health hazards.

NIOSH is focusing its evaluations of potential hazards in the recombinant DNA area of the biotechnology industry for a variety of reasons. First, the number of workers involved in recombinant DNA applications is potentially higher than any other segment of the biotechnology industry (e.g., in vaccine or antibiotic production). Industries outside the pharmaceutical sector are also applying recombinant DNA techniques. These industries which are involved in such areas as energy development and chemical production have far less experience in handling biological agents and may be less aware of potential or actual biohazards.

Also, recombinant DNA applications are in the preliminary stages of capitalization. Recommendations for safeguarding health can be most cost-effective if installed now, avoiding expensive retrofit. Rarely do we have such opportunities for active prevention in the early stages of development of potentially hazardous processes.

NIOSH is initiating a program to help define the potential for health hazards to workers participating in commercial recombinant DNA applications. We will continue to advise OSHA of our findings and recommendations.

As part of this program we will continue the on-site surveys of companies moving into scale-up or pilot production phases of operation, in order to assess the control measures and reduce human exposures. We are including evaluation of the following areas as appropriate:

1) physical containment design (e.g., fermenters)
2) engineering controls (e.g., ventilation, filter of exhaust gases)
3) work practices (e.g., operational protocols, clothing and hygiene requirements)
4) validation procedures (e.g., sterilization and fermenter containment)

5) emergency procedures (e.g., spills)
6) environmental monitoring
7) medical surveillance (e.g., serum samples for serologic changes; monitor possible physiologic changes related to product exposure)
8) worker education

It will be necessary to request directly from the companies proposing work, those submissions which have been made under the NIH voluntary compliance program (See Attachment). These submissions describe the design of the physical containment facilities, work practices and biological systems which are intended to ensure safe operation of the production process. As NIH can attest, adequate safety evaluation is impossible without this information.

In order to support our evaluation, we will seek information about fermentation processes which use similar equipment to that proposed for recombinant DNA fermentation application. Data obtained on fermenter reliability and effectiveness, accidents and possible routes of exposures should help us to determine whether potential health hazards in other biotechnology applications need assessment.

Appropriate medical surveillance has been discussed by different groups. The Recombinant DNA Advisory Committee has discussed the need for medical surveillance and NIOSH has been asked for recommendations by some of the companies we have visited. The varied nature of proposed host-vector systems and the multitude of possible biologically active products and byproducts make the determination of appropriate and effective medical surveillance a complex issue. To help address this need, NIOSH, together with other CDC experts in infectious disease, epidemiology and molecular biology will expeditiously develop guidelines for medical surveillance techniques.

It would be helpful if those companies proceeding with "large-scale" recombinant DNA applications inform NIOSH, so that the Federal agencies charged with the protection of public health will have basic information upon which to act in the event hazards unexpectedly emerge. This notification to NIOSH should at a minimum contain the name and location of the company, the host-vector system utilized, and the product which will be produced. The companies should also maintain internally complete records of their projects. The records should detail such information as the biological system, physical containment level, design and configuration, and identification of workers with their time associated with the projects. It is important to bear in mind that the retention of this type of information by the companies should not be unduly burdensome, because they would invariably be needed for the project development. This information would be necessary to evaluate any hazards which might unexpectedly emerge.

After the completion of the preliminary site-visit program described above, we are also proposing to study, as indicated by industry information, any particular projects which might have a higher potential for hazard or for guarding against it.

We estimate that NIOSH will publish general recommendations next year. Our recommendations will be based upon the assessments conducted and the current level of knowledge in this area. This publication will detail work practices and control technology which will minimize exposures to workers from potential hazards.

Attachments
cc: Members of Committee

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL

NATIONAL INSTITUTE FOR OCCUPATIONAL
SAFETY AND HEALTH
5600 FISHERS LANE
ROCKVILLE, MARYLAND 20857

MAY 6 1980

Dr. William J. Gartland
Director, Office of Recombinant DNA Activities
National Institutes of Health
Bethesda, Maryland 20205

Dear Bill:

As suggested to us by Bernie Talbot, I am formally requesting a copy of all submissions made to the National Institutes of Health (NIH), Office of Recombinant DNA Activities from private companies on large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules.

To assess hazards possibly associated with this new technology, the National Institute for Occupational Safety and Health (NIOSH), must be able to evaluate the technical information associated with "large-scale" work. The information submitted by industry to NIH is a necessary component, together with industrial hygiene surveys and control technology assessment, of a hazard evaluation.

You may wish to advise the private companies that under 29 U.S.C. 657B and by virtue of 29 U.S.C. 669B, NIOSH has the authority to request information needed to support its research mandate to explore new technology for occupational health and safety concerns. NIOSH would safeguard the submissions as stipulated under 29 U.S.C. 664.

Your cooperation has aided us in a difficult task and is greatly appreciated.

Sincerely yours,

Tony

Anthony Robbins, M.D.
Director

Statement

Subcommittee on Industrial Practices
Federal Interagency Advisory Committee on Recombinant DNA Research
June 18, 1980

Mr. Chairman and Members of the Subcommittee:

We appreciate this opportunity to meet with the Subcommittee to briefly discuss the subject of occupational health as it is related to the emerging and important field of recombinant DNA research and technological development. Accompanying me today is Dr. Ann Skalka from the Roche Institute of Molecular Biology. Dr. Skalka is a recognized expert in the field of recombinant DNA research. More importantly, she is a highly respected world authority on the subject of risk assessment,having served as the convenor and chairperson of the first Working Group on Risk Assessment of the Committee on Genetic Experimentation of the International Council of Scientific Unions. I shall make a brief presentation after which Dr. Skalka and I shall be pleased to respond to your questions.

It is important to place the subject of risk associated with recombinant DNA research and technology in proper perspective. The procedures used in recombinant DNA laboratory research and production are based upon well established techniques in the basic sciences of molecular biology, biochemistry, microbiology and engineering. There is nothing novel or unique in these routine procedures that would suggest the need for extraordinary precautions or special handling by laboratory personnel or supervisors. On the contrary, the enfeebled nature of host

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Presented by John G. Adams, Ph. D., Vice President, Office of Scientific and Professional Relations, Pharmaceutical Manufacturers Association cells and vectors as required by the biological containment provisions of the NIH Guidelines would suggest less stringent rules than are currently practiced in non-recombinant DNA microbiological research in which personnel are exposed to more potent and viable bacteria, viruses and other pathogenic organisms. We, nevertheless, support the safety provisions of the Guidelines as embodied in the biological and physical containment provisions including the section on laboratory practices and are committed to voluntary compliance with them. Dr. Skalka is prepared to respond to your questions concerning assessment of systems currently permitted under the Guidelines and their relationship to the protection and safety of laboratory personnel and the general public.

Some concern has been expressed about the increased risk to which laboratory and production personnel might be exposed in the scale-up of operations from laboratory to pilot plant to full industrial production. Dr. Irving Johnson, of Eli Lilly and Company, a firm with vast experience in fermentation technology upon which future industrial production of recombinant DNA materials will be based, will comment later on the industrial safety features of large-scale fermentation and associated process engineering. By way of preface to his comments, I merely wish to refer the Subcommittee to the general consensus as expressed at the recent meeting of the RAC and elsewhere that the relative risk of accidents in this field is probably greater in a research laboratory than would be the case in an industrial operation due to the nature of the equipment, and to the number of laboratories and research personnel involved. To the best of my knowledge and experience, the safety record of the industry in the production of antibiotics, steroids and other substances by fermentation has been outstanding. There is no reason to believe that the fail-safe systems employed in the engineering of such large-scale fermentation and processing equipment would be any less efficient than those employed in recombinant DNA operations. By comparison to other industrial processes, including the production of potent chemicals and biological products, the risk to workers and to the public of industrial recombinant DNA technology is probably minimal.

It is also important in assessing relative risk to consider the technical competence of those engaged in recombinant DNA research or production. Most of the research in this field is being conducted in laboratories that are supervised by persons with advanced scientific training. It is reasonable to assume that they are familiar with the nature of the organisms they employ and that their laboratories are in compliance with the NIH Guidelines, including the training of non-professional technical personnel and medical surveillance of all laboratory personnel. It is of interest and importance in this context to note that prior to the discovery of recombinant DNA techniques and the perceived need for guidelines, microbiological research and production of bacterial and viral vaccines was subject only to the commonly accepted principles of good laboratory practices, and in the case of production of biological products, to the recognized need for stringent self-imposed rules for physical containment, including the protection of scientists and other personnel engaged in such work. The safety record of these well-established procedures speaks for itself.

In concluding these brief remarks, I wish to emphasize that the member firms of the PMA, which I represent as well as other industrial firms engaged in recombinant DNA activities, endorse all of the provisions of the NIH Guidelines and to the best of my knowledge have voluntarily complied with them. Such compliance includes physical and biological containment, training of personnel, good laboratory practices, medical surveillance, the establishment of Institutional Biosafety Committees and all other factors necessary to assure the health and safety of persons engaged in research and/or production. We deem such compliance to be in the public interest and for that matter in our own best interest. We commend the National Institutes of Health for their foresight in devising the Guidelines to assure that the benefits to be derived are not impeded by unnecessary constraints in research and industrial applications of this technology. We also applaud the creation of this Subcommittee on Industrial Practices as a means for consideration of the special problems which may arise as commercial development of this technology becomes a reality. I can provide you an assurance that the industrial sector is prepared to offer its expertise and assistance as you undertake the assignment given to you by the Federal Interagency Advisory Committee.

Page 1 of Attachment E

THE COMMITTEE ON GENETIC EXPERIMENTATION
A SCIENTIFIC COMMITTEE OF THE INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS

First Report to COGENE
from
The Working Group on Risk Assessment

A. M. SKALKA (USA) Convenor
G. BERNARDI (France)
V. SGARAMELLA (Italy)

July 1978

A Description of COGENE

The parent body of the Committee on Genetic Experimentation (COGENE) is the International Council of Scientific Unions (ICSU), an international non-governmental scientific organization of 18 autonomous international Scientific Unions and more than 60 National Members, i.e. academies of science, research councils or similar scientific institutions.

COGENE was created by decision of the 16th General Assembly of ICSU and came into being in 1977. It is a Scientific Committee, one of a type formed by ICSU whenever a task is defined that is of major interest to several unions and the program is of a long-term nature. COGENE is supported by seven member unions — Pure and Applied Chemistry, Biological Sciences, Biochemistry, Pure and Applied Biophysics, Nutritional Sciences, Pharmacology and the Immunological Societies — and includes a number of scientists prominent in the field of recombinant DNA research, which is currently the major focus of interest of COGENE and the reason why the Committee came into being.

Membership

A.A. Bayev (U.S.S.R.)
G. Bernardi (France)
S.N. Cohen (U.S.A.)
A.F. Langlykke (U.S.A.)
K. Murray (U.K.)
N. Notani (India)
R. Riley (U.K.)
A.M. Skalka (U.S.A.)
C. Steinberg (Switzerland)
J. Tooze, Secretary (BRD)
I. Watanabe (Japan)
W.J. Whelan, Chairman (U.S.A.)
E. Wollman (France)

Observers have been appointed by FAO, ICRO, UNEP, UNESCO and WHO. Twenty-one nations have appointed National Correspondents.

Purposes and Objectives

The terms of reference of COGENE include the following purposes and objectives:

COGENE is a Scientific Committee of ICSU, established to serve as a nongovernmental, interdisciplinary and international council of scientists and as a source of advice for the benefit of governments, inter-governmental agencies, scientific groups, and individuals, concerning recombinant DNA activities.

Among its purposes shall be:

a) to review, elvaluate and make available information on the practical and scientific benefits, safeguards, containment facilities and other technical matters,

b) to consider environmental, health-related and other consequences of any disposal of biological agents constructed by recombinant DNA techniques,

c) to foster opportunities for training and international exchange, and

d) to provide a forum through which interested national, regional and other international bodies may communicate.

OGENE shall also consider, if necessary, other related activities which nay give rise to public concern.

Current Activities

COGENE currently has three Working Groups active in the areas of:

a) Guidelines for Research on Recombinant DNA. The Convenor is S.N. Cohen (U.S.A.). The Group will make its first report in September 1978.

b) Risk Assessment Experiments. The Convenor is A.M. Skalka (U.S.A.). This is the Group's first report.

c) Benefits and Applications of Recombinant DNA. The Convenor is W.J. Whelan (U.S.A.).

COGENE and the Royal Society (Great Britain) will convene an international conference on recombinant DNA at Wye College (University of London) Kent, U.K. during 1-4 April 1979.

SUMMARY

The Working Group has been charged with the task of gathering information related to assessment of risks associated with recombinant DNA research. The information in this report was obtained by observation and participation at a number of meetings and hearings, by survey of the literature and by direct inquiry to national and international organizations, and individual scientists.

Our analyses indicate that the concerns regarding recombinant DNA research can be summarized as follows:

1. Organisms carrying recombinant DNA may spread in the natural environment and disrupt existing ecological equilibria.
2. These organisms might produce some toxic or noxious substance, or otherwise cause disease.
3. By exploiting this technology, scientists may be crossing some hypothetical barrier to DNA exchange between eukaryotes and prokaryotes and thus affect pathogenicity or dispersion of pathological agents.

Reports from the Falmouth Meeting (section IV) and COGENE-sponsored analyses of *E. coli* K-12 systems (section VI) show that many relevant experiments have already been conducted by scientists in such closely related fields as epidemiology and infectious diseases. Their observations that *E. coli* K-12 has a limited prospect of survival and their consensus that this bacterium cannot be converted into an epidemic pathogen by laboratory manipulations with DNA inserts, do much to satisfy the first concern mentioned above. In addition (section VII), long-term monitoring of laboratory workers who routinely handled K-12 organisms carrying transmission-proficient plasmids showed no bowel colonization despite the fact that the work was carried out without any special precautions. If the organisms which carry recombinant DNA cannot spread in the natural environment, then clearly the other concerns are also diminished. The conclusion of a NIH/EMBO Virology Workshop (section V) was that recombinant organisms carrying viral inserts cannot be any more hazardous than the viruses themselves, and in some instances may provide an opportunity to work more safely with virulent agents.

As will be seen in the report, important information on the nature and possible consequences of the manipulations used in recombinant DNA technology comes from experiments in a variety of fields. For example, attempts to understand the biological role of restriction enzymes have revealed that prokaryotic and eukaryotic DNAs can recombine *in vivo.* Other studies, including those with *Agrobacterium tumefaciens* and plant cells, also show that in some cases there is no natural barrier to exchange and expression of genes across the hypothetical prokaryotic-eukaryotic barrier. On the other hand, studies of animal virus genomes and eukaryotic genes for differentiated functions have revealed previously unsuspected possible barriers to their expression in

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prokaryotic backgrounds. It is clear that further studies of the molecular genetics, pathogenicity, ecology and other properties of organisms will continue to provide information relevant to the assessment of risk and that this source is no less important than specifically targeted experiments.

Replies to our Questionnaire (sections I and II) indicate that there are several projects currently underway directed specifically towards risk assessment. These include the two separate polyoma virus experiments currently sponsored by EMBO and by NIH and several NIH contracts aimed at testing and verification of EK2 and EK3 systems. Preliminary results, as well as conclusions from the NIH-EMBO Virology Workshop indicate that these studies are unlikely to reveal unknown hazards. These and other experiments which are aimed at elucidating various aspects of the ecology and natural history of microorganisms should provide useful information and will be important in the development of host-vector systems other than those based on *E. coli* K-12.

In summary, our analyses have revealed no scientific findings to justify any of the three concerns listed above; no risk unique to recombinant DNA research has been identified. Available evidence indicates that recombinations of the type made possible by this new technology can occur in Nature. Evaluation of *E. coli* K-12 shows that this bacterium is essentially harmless and that insertions of segments of foreign DNA into its genome cannot alter this property. With few exceptions, it seems likely that the same will prove true of other host bacteria. These conclusions cannot be ignored if the guidelines governing recombinant DNA research are to remain rational and useful.

Part I of the following report contains a detailed summary of information we have gathered and a selected bibliography. Part II includes source materials, documents received in reply to our questionnaire and the texts of reports which may not be readily available.

CONTENTS — PART I

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Section I	Questionnaire sent to various nations and organizations and record of responses	1
	1. Letter and questionnaires	1
	2. Tabulation of responses	2
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	1. Assessment of the adequacy of various forms of physical containment designs and equipment	7
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	6. Environmental impact of recombinant organisms	12
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Section III	Summary of WHO — Sponsored survey of individual scientists	15
Section IV	Report on the NIH — Sponsored Risk-Assessment Meeting at Falmouth on June 22, 1977	16
Section V	Comments on other relevant actions, reports and meetings	21
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CONTENTS — PART II*

Section I	Letters and Documents received in response to Questionnaire and follow-up letter (See Tabulation, Section I, Part 2, for cross references)
Section II	Materials received in connection with the Falmouth Risk Assessment Meeting
Section III	(1) Testimony of Philip Handler, President of the U.S. National Academy of Sciences, before a U.S. Senate Subcommittee Hearing (Nov. 2, 1977) (2) Report of a U.S. National Academy of Sciences Panel on Risks and Benefits of Recombinant DNA Research Performed under the NIH Guidelines (Nov. 1, 1977)

* Part II is available from COGENE (c/o Dr. W.J. Whelan) P.O. Box 016129, Miami, Fl. 33101, U.S.A. at a cost of $20.

Section I
QUESTIONNAIRE AND RESPONSES

1. LETTERS SENT TO NATIONS OR ORGANIZATIONS ON MAILING LIST

COGENE, a scientific committee of ICSU (International Council for Scientific Unions), has been established to deal with issues arising from the advent of recombinant DNA research. Among its purposes and objectives (see enclosed statement) is a consideration of environmental, health-related and other consequences that might result from the accidental dispersal of biological agents constructed by recombinant DNA techniques.

Evaluation of the considerable and wide-spread speculation, claims and counter claims, about the nature and magnitude of the risks associated with recombinant DNA research, is difficult without specific scientific information bearing on these questions. Consequently, one of COGENE's initial efforts is to gather information about what investigations are being undertaken to assess the risks of recombinant DNA experimentation.

Specifically, would you inform us if your national committee or scientific body is presently conducting or plans to carry-out experiments, of if you are aware of any organization or laboratory which is planning or conducting experiments to:

1. *Assess the adequacy or comparative advantages of various forms of physical containment designs and equipment;*
2. *Assess the adequacy or comparative advantages of various forms of biological containment;*
3. *Estimate the frequencies of pro- and eukaryotic genetic exchange in Nature (i.e. How widespread and frequent are foreign DNA's taken up, and maintained by pro- and eukaryotic organisms?);*
4. *Determine whether toxic recombinants (e.g. organisms bearing botulinum, tetanus or diptheria toxin genes) can be constructed and/or whether "shotgun" experiments using DNA from various sources can produce lethal infections, tumors, etc.*
5. *Develop means for monitoring laboratory personnel for exposure to or infection by recombinant organisms or vectors;*
6. *Explore the potential impact of recombinant organisms on nonhuman species (e.g. animals, plants), the atmosphere and oceans;*
7. *Clarify aspects of the ecology and natural history of microorganisms, plasmids and bacteriophages as they relate to the probabilities of disseminating infections via such vectors;*

If you would summarize any plans, results or provide reports, protocols, etc. concerning experiments of the type described above it would be extremely helpful to us.

There is some urgency in gathering this information and your earliest reply would be greatly appreciated.

COGENE plans to summarize the "world-wide" experiences of risk-assessment research and such summary would certainly be made available to each group responding to this letter.

Sincerely yours,

A.M. Skalka, Ph.D.
for the Working Group on Risk Assessment
A.M. Skalka (USA) Convenor
G. Bernardi (France)
V. Sgaramella (Italy)
C. Steinberg (Switzerland)

On December 7, 1977, a second request for answers to the questionnaire was sent to those nations or organizations who had not responded to our letter of September 22, 1977.

2. TABULATION OF RESPONSES

Twenty-one of twenty-five nations or organizations contacted responded to our request for information. Following is a tabulation which lists the nation or organization, and the contact or responder. The document numbers identify the letters and other information which was received and are reproduced in Part II of this report.

TABULATION OF RESPONSES

Nations or Institutions Contacted	Contact or Responder	Reply to Questionnaire (Document Number)
Australia	Dr. G. Ada Department of Microbiology John Curtin School of Medical Research Australian National University Canberra ACT 2600 Australia	D-6
Austria	Professor Hans Tuppy Institut für Biochemie University of Vienna A-1090 Vienna Wahringerstrasse 17 Austria	No reply

Belgium	Professor W. Fiers Advisory Sub-Group-Belgian Committee on Medical Ethics Laboratory of Molecular Biology Rijksuniversiteit Gent Beukendreef 3 9120 Destelbergen Belgium	D-10
Canada	Dr. F.S. Rolleston Director-Special Programs Medical Research Council Ottawa K1A OW9 Canada	D-4
Denmark	Dr. S. Guldborg Danish Research Administration Forskningssekretariatet Holmens Kanal 7 1060 Copenhagen Denmark	D-18
Ghana	Professor E. Laing University of Ghana P.O. Box 25 Legon, Accra Ghana	No reply
France	Dr. F. Miquel Commission de Controle pour les Manipulations Genetiques in Vitro D.G.R.S.T. 35 rue Saint Dominique 75700 Paris, France	Oral (G. Bernardi) 4/3/78
Germany (W)	Dr. P.H. Hofschneider Max-Planck-Institute fur Biochemie 8033 Martinsried Bei Munchen Durchwahl 8585-292 Federal Republic of Germany	D-14
Ireland	Professor J.W. Harman Biomedical Genetics Committee c/o Medical Research Council of Ireland 9 Clyde Road Dublin 4 Ireland	No reply

Israel	Professor L. Sachs Chairman-Israel National Committee on Recombinant DNA The Weizmann Institute of Science Rehovot, Israel	D-21
Italy	Dr. A. Falaschi Laboratorio de Genetica ed Evoluzinistica National Research Council via Sant'Epifanio 14 27100 Pavia, Italy	Oral (V. Sgaramella) 4/3/78
Japan	Dr. T. Iino Laboratory of Genetics The University of Tokyo Hongo, Tokyo 113, Japan	D-19
Netherlands	Dr. A.W.J.J. Buija Royal Netherlands Academy of Sciences Kovenierburgwal 29 Amsterdam The Netherlands	D-25
New Zealand	Dr. E.G. Bollard Interim National Correspondent COGENE The Royal Society of New Zealand P.O. Box 12-249 Wellington, New Zealand	D-9
Norway	Professor S. Laland Norwegian National Committee for Recombinant DNA Research c/o N.A.V.F. Munthesgate 29 Oslo 2, Norway	No reply
South Africa	Professor O.W. Prozesky National Institute for Virology Private Bag X4 Sandringham 2131 Johannesburg, South Africa	D-7
Sweden	Marianne von Glehn Secretary of the Committee of Questions concerning research with R-DNA. Statens Naturvetenska Pliga Forskningsrad Swedish National Science Research Council Box 23 136 104 35 Stockholm 23-Sweden	D-11

Switzerland	Professor W. Arber Chairman-Commission for Experimental Genetics of the Swiss Academy of Medicine Department of Microbiology Biozentrum Klingelbergstrasse 70 4056 Basel Switzerland	D-5
United Kingdom	Professor M.H. Richmond University of Bristol Dept. of Bacteriology The Medical School University Walk Bristol BS8 1TD United Kingdom	D-12
U.S.A.	Dr. DeWitt Stetten, Jr. Deputy Director for Science Office of the Director National Institutes of Health Bethesda, Maryland 20014 U.S.A.	D-13, 16, 17
U.S.S.R.	Professor A.A. Bayev Institute of Molecular Biology Academy of Sciences of the USSR Vavilov Street 32 117312 Moscow B 312 USSR	D-24
EMBO	Dr. John Tooze EMBO Postfach 102240 69 Heidelberg 1 Federal Republic of Germany	D-1
	and	
	Dr. K. Murray and Dr. B. Klein EMBO 69 Heidelberg-1 Postfach 102240 Federal Republic of Germany	D-8
	Dr. C. Weissman, Chairman Standing Advisory Committee on Recombinant DNA Institut for Molekularbiologie 1 Universität Zurich Honggerberg CH-8049 Zurich, Switzerland	Responded, through EMBO, see D-1

WHO	Dr. K. Bögel Special Program on Safety Measures in Microbiology World Health Organization 1211 Geneva 27, Switzerland	D-3, 23
NAS	Dr. Maclyn McCarthy, Chairman N.A.S. Committee on Laboratory-Created Biohazards The Rockefeller University 66 Street and York Avenue New York, New York, 10021	D-2

Section II
ANALYSIS OF RESPONSES

Questions (1) and (2):

The questions were whether national committees or scientific bodies are presently conducting or plan to carry-out experiments, or if adressees are aware of any organization or laboratory which is planning or conducting experiments to:

(1) *Assess the adequacy or advantages of various forms of physical containment designs and equipment;*

(2) *Assess the adequacy or comparative advantages of various forms of biological containment.*

Comments:

Seven of the twenty-five answers were positive with regard to both questions.

(i) WHO (Document No. 3) has an ongoing special program on Safety Measures in Microbiology. It is presently active through four working groups, whose terms of reference are:
  (a) maximum containment laboratories (question 1 of our questionnaire);
  (b) development of emergency services;
  (c) laboratory safety elements (which could, in principle, relate to question 2 of our questionnaire, but actually does not). The response from Mr. V.R. Oviatt, chairman of this working group, is considered below;
  (d) transportation of infectious substances.

(ii) Mr. V.R. Oviatt (Document No. 23) of DHEW/NIH, and Chairman of the WHO Working Group on Laboratory Safety Elements describes the initial activity of this group, and among other items, lists laboratory safety equipments and laboratory safety facilities as intrinsic components of the program. No mention is made of biological containment. A detailed report is expected following the WHO meeting in Dusseldorf, at the beginning of April 1978.

(iii) Dr. M.H. Richmond of UK/GMAG (Document No. 12) stresses the desirability of greater concentration among the various initiatives with the same general goals as COGENE. In particular, Question 1 is close in scope to the EMBO/NIH Workshop of March 1977 (see Bibliography Section), of which the unpublished report should be made available to this working group. In addition, an analysis of the relative protective value of the various levels of containment has been carried out; design and specification of protection and laminar flow cabinets are being scrutinized; the Microbial Research Establishment (MRE), Porton Down, England is continuously involved in the evaluation of physical containment; finally a Monograph (No. 6) of the Public Health Laboratory Service is available from Her Majesty's Stationery Office: it contains a list of papers (up to 1973) related to the problem of handling pathogens.

As for Question 2, tests are planned in the U.K. to investigate attenuated strains of *E. coli* K-12, as well as thermophilic bacteria and *B. subtilis*. Also vectors (phage and plasmids) are being examined specifically for use in biologically contained experiments. No official action is considered, and progress in this field is expected to depend mainly on U.S. initiatives (e.g. Falmouth Workshop, or NIH action).

(iv) Dr. S. Rolleston, of CANADA/MRC (Document No. 4) has informed us that a Sub-committee on Performance Standards has been established to determine criteria for containment equipment and mechanisms for testing of such equipment *in situ* in the lab. A report is expected (Oct. 3, 1977). "None" is the answer to Question 2.

(v) Dr. D. Stetten of U.S. DHEW/NIH, (Document No. 13) has submitted answers to both questions:

1. Dr. E. Barkley, director of Research Safety, NCI (Document No. 13) mentions that the performance capabilities of safety cabinets are being evaluated at the NCI: their performance could be extrapolated from cancer research to genetic manipulation. A contract to the Naval Bioscience Laboratory of UC in Berkeley by NCI aims at studying biohazards of lab operations e.g. associated with aerosols, worker techniques, etc.

   NIH involvement with P4 facilities is in general directed at the objective of Question 1.

2. NIH is supporting a variety of studies on biological containment (Document No. 13): the relevant contracts are administered by NIAID. They aim at (a) verification of genotype and phenotype of hosts and vectors, (b) testing vector-host systems to confirm containment, shown by laboratory studies, in relevant environments including animals and man.

   Quarterly reports are produced by the contractors, and COGENE might take advantage of them, or of some elaboration of them made available by ORDA.

   A report signed by Dr. W. Szybalski to the Recombinant Advisory Committee from the Lambda Phage Working Group was enclosed by NIH: it outlines standardized EK3 tests and other items (A.M. Skalka was present at the meeting and is a coauthor of the report.). A memorandum from Dr. M. Martin to the NIH Recombinant DNA Molecule Program Advisory Committee on endorsement of the risk assessment experiment with cloned polyoma is also pertinent (Document No. 13): its goal is to assess the factors (bacterial vector and animal host) that determine if a DNA molecule can be transferred intact from *E. coli* host into mammalian cells. Broader scopes are proposed in other tests, involving shot-gunning of yeast, *Dictyostelium*, *Salmonella* and *Drosophila* DNA into *E. coli* K-12 and the study of possible alterations of its pathogenicity in mice.

(vi) Dr. J. Goodman of SAI (Science Applications Inc., Ca.; Document No. 15) offers the professional collaboration of his organization, specialized in risk assessment efforts related to atomic energy facilities.

(vii) Dr. D.F. Liberman, of MIT Biohazards Assessment Office (Document No. 16) describes procedures to test the escape of non-lytic and lytic EK-1 and EK-2 bacteriophage systems from primary physical containment. They are explicity directed at the problem of environment and personal monitoring, and designed to provide information concerning the effectiveness of containment under laboratory conditions.

In summary, the answers which we received and those analyzed in this report confirm that both the USA and UK are carrying on studies specifically directed at the evaluation of physical and biological containment. WHO is aiming at a broader objective, safety in all microbiological laboratories, through a reappraisal of all the relevant factors.

Question 3:

Estimate the frequencies of pro- and eukaryotic genetic exchanges in Nature (i.e. how widespread and frequent are foreign DNAs taken up, and maintained by pro- and eukaryotic organisms?).

Comments:

Concerning Question 3, pro- and eukaryotic genetic exchanges in Nature, we have distinguished the following four parameters:

a. uptake by pro- or eukaryotic cells of foreign DNA:
b. integration of foreign DNA into the host cell genome;
c. peristence or maintenance of foreign DNA in the host cell genome;
d. expression of foreign DNA.

Concerning point a. there is ample evidence in the literature that DNA of almost any origin can be taken up both by bacterial and eukaryotic cells.

As for point b., integration of foreign DNA is clearly demonstrated in several instances; 1) integration of a piece of plasmid DNA from *Agrobacterium tumefaciens* into plant genomes causes tumor formation in plants (data of Schell and Van Montagu and other cited in our bibliography and by Dr. W. Fiers of Belgium; (Document No. 10). 2) Integration of eukaryotic DNA into a prokaryotic genome (S.N. Cohen cited in our bibliography). In other cases, integration is demonstrated indirectly by the expression of the foreign DNA (see below under d).

It seems reasonable to assume that persistence of foreign DNA in the prokaryotic or eukaryotic host genome, point c., will require selective pressure. Such persistence is clearly demonstrated by the expression of foreign DNA.

Expression of foreign DNA, point d., is well documented in the case of yeast and *Neurospora* genes (R. Davis, J. Carbon, and others) able to complement nutritional deficiences in *E. coli*. The production of human chorionic gonadotrophin by bacteria isolated from cancer patients (quoted by Adelberg — Document No. 13) might represent, if confirmed, another instance of expression of eukaryotic genes in bacteria. It should be pointed out, however, that the complex processing, involving RNA splicing, which has been found in several genes from animals makes it very unlikely that a correct expression of such genes may take place in bacteria.

We note that the expression observed to date has been with genes of lower eukaryotes in prokaryotes. The ability to be expressed may reflect: (1) their closer phylogenetic relatedness, (2) the fact that larger gene samples must be tested for more complex eukaryotes or (3) a basic difference between genes of metabolic pathways (for some of which expression has been documented) and genes of differentiated function (so far shown to contain inserts which must be processed in futher steps and are not present in mature mRNA). Under experimental conditions there is, in theory, no limit to the exchange of DNA between eukaryotes and prokaryotes. The effectiveness and biological significance of such exchanges will be determined not by whether the DNA can be exchanged, but by factors determining whether or not it can persist in the host-cell and be expressed in it.

Question 4:

Determine whether toxic recombinants (e.g. organisms bearing botulinum, tetanus or diptheria toxin genes) can be constructed and/or whether "shotgun" experiments using DNA from various sources can produce lethal infections, tumors, etc. . . .

Comments:

Concerning question 4, the construction of toxic recombinants appears to be quite possible for prokaryotic toxin genes cloned in prokaryotes, and rather unlikely for eukaryotic toxin genes cloned in prokaryotes for the reasons given in discussion of Question 3 (see also discussion of Question 7).

Whether "shotgun" experiments using DNA from various sources can eventually result in dangerous infections, etc. . . . is not yet clear, but is conceivable in some cases, for example, when manipulations are made with dangerous pathogens. Clarification of such questions is the stated objective of the "polyoma" type experiments sponsored by EMBO and NIH and described by Drs. J. Tooze (Document No. 1) and M. Martin (Document No. 13). Until experience dictates otherwise, it seems reasonable that organisms carrying recombinant DNAs should be handled under the conditions appropriate for the most hazardous of the recombinant's components.

Question 5:

Would you inform us if your national committee or scientific body is presently conducting or plans to carry-out experiments, or if you are aware of any organization or laboratory which is planning or conducting experiments to:

"Develop means for monitoring laboratory personnel for exposure to or infection by recombinant organisms or vectors".

Comments:

Three countries provided us with comments regarding efforts to devise monitoring procedures (U.K., U.S.A. and the Netherlands):

(i) Dr. M. Richmond (UK/GMAG, Document No. 12) reports that machinery for routine epidemiological monitoring is under discussion by the UK/GMAG which is considering regular and frequent monitoring only only for those involved in category IV (P4) work.

Emphasis is being given to the development of strains bearing suitable "markers" (e.g. 5-fluorouracil resistance) other than drug resistance involving commonly-used antibiotics such as tetracycline.

(ii) Dr. D. Liberman, an NIH contractor (Document No. 16) describes efforts to develop techniques for environmental and personnel monitoring. Methods of air sampling to test for lytic bacteriophages seem feasible. Dr. Liberman notes that the need (if any) for monitoring will be determined by the Institutional Biohazards Committee with appropriate methods incorporated into individual projects. He cites as an example a project involving study of insulin genes. In this case, arrangements have been made for a local research foundation to perform blood sugar and blood insulin determinations on a routine basis for all personnel associated with the project.

(iii) Dr. R. Curtiss (USA, Document No. 17) suggests that in some cases it might be appropriate to take nasal, facial and wrist swabs at the end of a work day and to perform quantitative tests for the presence of hosts and/or vectors containing suitable "markers". Curtiss states that such tests should prove negative if the individual uses all of the accepted microbiological practices and safety equipment, and thus would be a spot-check on carelessness or perhaps would be useful to verify accidental exposure. He cites, as do others (see Petrocheilou and Richmond in bibliography), the negative results obtained to date from faecal samples.

(iv) In the Netherlands (see Document No. 25), efforts are underway to develop selective tests for identification of *E. coli* K-12 strains used in recombinant DNA experiments.

In general, it appears that the U.S. approach has been to develop systems (EK1, EK2, EK3 — See Document No. 13) which can be tested and certified in a standardized way. Systems which prove safe in such "worse-case" tests should not require constant monitoring, except for the relevant enfeebling phenotypes, especially when used in conjunction with physical containment equipment and facilities, the efficacy of which can be independently verified.

Question 6:

"Explore the potential impact of recombinant organisms on non-human species (e.g. animals, plants), the atmosphere and oceans."

Comments

Only a few of the 25 answers received have a direct bearing on this question. Some touch it marginally as described below:

(i) Dr. M.H. Richmond, of UK/GMAG (Document No. 12), indicates that although no work can be specifically identified as targeted to this point, a subcommittee is looking into the possible hazards to plants.

At present, advice is being given that (a) plants used as hosts for novel recombinant DNA should require special containment in suitable greenhouses; (b) the most likely significant hazards would arise from handling fungi with aerial spores; conventional containment would be inappropriate.

(ii) Dr. E.G. Bollard, from New Zealand GMAG (Document No. 8) stresses the specific interest of his country in experimentation with plants. Reportedly, attempts have been made to introduce N_2 fixation genes into mycorrhizal fungi. The New Zealand guidelines (which are based on the U.K. William's Report) emphasize the need for containment measures with experiments involving plants, and their requirement to be commensurate with current plant quarantine practice.

Protoplast fusion when done with cells from flowering plants belonging to different families, requires the category I (UK) containment level. Fusion with micro-organisms, including algae, may need at least category II containments.

(iii) The WHO Special Program on Safety Measures in Microbiology (Document No. 23) is considering the potential impact of "recombinant" organisms on targets other than man. The report of WHO/NIH consultations on Facilitation and Safety in the International Transfer of Research Material is aimed at preventing health as well as ecological offenses by any potentially infectious organisms. The same applies to the broader objective of WHO action toward the elaboration of safety measures in microbiology.

(iv) Dr. D. Stetten of NIH (Document No. 13) relates his answer to the broader goal of risk assessment efforts. As bearing on this point, he cites the "polyoma" experiment as well as the other experiments (shotgunning of variety of eukaryotic DNAs in *E. coli* K-12 followed by infection of normal and germ-free mice), directed at testing possible changes in pathogenicity of *E. coli* K-12.

> The effects on mice following infection with *E. coli* cells carrying the cDNA's to C-type virus should give concrete information on the fear of inadvertent cloning of retrovirus genomes in the course of shot-gun experiments with mammalian DNAs. At a later stage, cloning or replication-defective murine-sarcoma viruses could be studied, and attempts could be made to induce carcomas in mice with such clones.
>
> Finally, shot-guns of chromosomal DNA from mice known to contain C-type viruses could provide much wanted information. Although not cited by Dr. Stetten, the NIH Environmental Impact Statement can be considered: the possible undesirable impact of experiments conducted under the guidelines, is concluded to be extremely unlikely, and difficult to evaluate in realistically meaningful terms (see Holliday's paper listed in Bibliography section).

The alteration of the biological properties of useful animals or plants by the introduction of recombinant DNA molecules will certainly involve a series of specific experimental steps. Each will be designed to overcome one or more of the series of barriers in the recipient organisms and, of course, the experiments will require appropriate physical containment until the recipient organism has been thoroughly characterized. We consider the probability of accidentally altering the biology of plants and animals outside the laboratory to be in practice negligible.

Question 7:

"Clarify aspects of the ecology and natural history of microorganisms, plasmids and bacteriophages as they relate to the probabilities of disseminating infections via such vectors."

Comments:

There is some overlap with these responses and answers to Question 3. Thus:

(i) Dr. Bollard (New Zealand; Document No. 9) cites experiments of Professor Bacon (Massay University) which are testing the ability of chloramphenicol-resistant coliforms isolated from sewage to transfer drug resistant plasmids to *E. coli* K-12. It is hoped that these results, as well as data from W. Arber (Switzerland; Document No. 5) regarding incorporation of insertions or deletions into Pl plasmids, will be available in the not-too-distant future.

(ii) Dr. T. Iino (Japan; Document No. 19) reports that the "distribution of R-plasmids and their transferability in the natural environment", is being studied and that Professor S. Mitsuhashi, Gumma University, is the Convenor of the relevant research group sponsored by the Japanese Ministry of Education, Science and Culture.

(iii) Dr. Buija (Netherlands; Document No. 25), commenting for the "Committee in Charge of the Control of Genetic Manipulation" describes two projects relevant to this question. The goal of the first, under Professor D. Tempest, Laboratory of Microbiology, University of Amsterdam, is study of the persistence of R-plasmids, including plasmids used as vectors in recombinant DNA experiments, in various environmental conditions. The second project, under Dr. P.A.M. Guinne, Laboratory for Bacteriology, National Institute of Public Health, Bilthoven, is aimed at developing selective tests for identification of *E. coli* K-12 strains used in recombinant DNA experiments.

(iv) Professor M. Richmond (UK; Document No. 12) comments for GMAG that specific programs have not been initiated in this area but there is much on-going research relative to the topic covered in this question as regards man as well as animal and plant populations. Examples of reports from such studies were included as bibliography and appendices in a reply from Dr. S. Falkow (USA; Document No. 22).

(v) Dr. Falkow (together with Dr. P. Shipley) is currently preparing a comprehensive review on "the role of plasmid-mediated enterotoxins and specific adhesive properties" as they relate to dissemination of infection, and a copy of the review will be made availalbe to our Working Group when it is completed.

We note that most Guidelines governing recombinant DNA research forbid manipulation of genes specifying toxins, despite the fact that analogous exchanges probably occur naturally as exemplified by the immunological relatedness of enteric and cholera toxins (Falkow, S., Lorenzini — WHO Symposium, in press). Conversely, as Dr. M. Richmond has commented (Document No. 12), it is unknown whether there are any likely circumstances in which recombinant DNA could add to the hazard intrinsic in an already infective organism.

We conclude that in the next few years we shall learn much more about the natural history of plasmids, their exchange among bacterial species and their relationship to disease, and we anticipate that this new knowledge will be relevant to risk assessment in these as well as the bacteriophage-based systems. Knowledge to date indicates the safety precautions in all present Guidelines are more than sufficient to ensure safety (i.e. use of nontransmissible plasmids and *E. coli* K-12 based systems). No doubt future results will dictate adjustment of these measures where appropriate.

Section III
SUMMARY OF WHO — SPONSORED RISK ASSESSMENT SURVEY

During January of 1976, the WHO performed a direct survey of the opinions of scientists knowledgeable in this area of biological research. In a letter circulated to over two hundred of them, suggestions were requested on (1) which kind of experiments, if any, could provide useful information, (2) the facility suitable to perform them, (3) the resources and time required.

Of the sixty or so answers received, a handful expressed opposition to this WHO action, on the grounds that (a) it was duplicating efforts of other organizations, (b) risk evaluation would yield no useful information, (c) the risks were negligible, or (d) it was inappropriate for WHO to take initiatives in this area.

Among the many (over 90%) positive replies, some expressed the opinion that the broad field of the microbial and plasmid ecology was one from which much could be learned. Studies involving feeding of specifically marked *E. coli* strains to volunteers, and monitoring of laboratory workers involved in plasmid experiments have been suggested by many scientists.

Several variations of the "polyoma" experiments currently being undertaken by EMBO and NIH have been described. In line with the proposal, some scientists have remarked that the most useful experiments for the evaluation of risks are exactly those for which a ban has been asked (e.g. the cloning in *E. coli* of genes for toxin formation). The study of the effect of foreign genes on the "fitness" of their hosts in a variety of media or environments, has also been presented as an important approach to evaluate the chance of survival of manipulated bacteria, were they to escape from the laboratory. The occurrnce of interspecific transfer of DNA was also considered a key element for the evaluation of risk, as was the knowledge of the stability of genetic information in recombinant DNA molecules and in fused cells. The probability of exchange of genes between micro-organisms and animal hosts should be studied under conditions where such exchange could be favored (as in a rotting carcass), or even required for survival: the example has been made of tissue culture cells exposed to toxic amounts of mercury in the presence of bacteria containing a plasmid capable of detoxifying the metal.

As for the feasibility of such experiments, the high-risk facilities of the Microbiological Research Establishment in the UK and the soon-to-be-ready laboratories of NIH in the US have been suggested by some. Budget evaluations, provided by several scientists, have given hints that most of these experiments need not be expensive, and could be done in a relatively short time (a few years at most).

Section IV

REPORT FROM COGENE'S OBSERVER AT THE FALMOUTH WORKSHOP ON RISK ASSESSMENT

The following report was sent on June 24, 1977 in the form of a memorandum to members of the COGENE Working Group on Risk Assessment, from Dr. A.M. Skalka, who attended the Workshop as an official observer for COGENE. Although informal, it received rather wide distribution owing to the delay in publication of the more formal report (to be published in the Journal of Infectious Diseases, May 1978) and the need for information on this subject.

A workshop on Studies for Assessments of Potential Risks Associated with Recombinant DNA Experimentation, sponsored by the National Institutes of Allergy and Infectious Disease and the Fogarty International Center of the National Institutes of Health, was held on June 20-21, 1977 in Falmouth, Mass.

The stated objectives of this meeting as outlined in the letter of invitation which is included in the material attached, was to concentrate on five major areas.

1. *Consideration of the possibility that there would be a change in the biology of* E. coli *K-12 after incorporation of a random piece of foreign DNA from various eukaryotic sources.*
2. *Consideration of changes brought about by the insertion of animal virus DNA.*
3. *Consideration of the biology and pathogenicity of* E. coli *K-12 not carrying recombinant DNA molecules.*
4. *Consideration of maintenance and spread of plasmids of* E. coli *K-12 in* vivo.
5. *Consideration of the "hypothetical barrier" which has been proposed to prevent the uptake of eukaryotic DNA by prokaryotic cells.*

As indicated by the schedule of topics and speakers for the meetings, these various areas were not considered in turn, rather the conference started- with a discussion of the consideration of the biology of E. coli *and the relevant host defenses. Of considerable interest, were a series of experiments presented by Dr. S. Formal, in which exhaustive attempts were made to induce pathogenicity in* E. coli *by deliberate insertion of a number of genes from* Shigella flexneri. *The results from all these attempts (which took place over several years) were essentially negative. Thus, even in this well-defined system, where assays for pathogenicity and virulence were available and conscious attempts were made to introduce genes that would render* E. coli *pathogenic, no success could be reported. These data together with the fund of knowledge already available from the infectious disease and epidemiology experts present, led to* *the unanimous conclusion that it would be virtually impossible to turn* E. coli *K-12 into a pathogen by the random insertion of a bit of eukaryotic DNA.*

Data presented which related to the epidemiology of E. coli *infections both in the gut and urinary tract, etc., indicated that the majority of all* E. coli *infections originate from the gut. That is,, the intestine is the most important reservoir of pathogenic* E. coli. *Consequently, the ability to colonize the gut is one of the most important parameters to measure for any* E. coli *strain in assessment of risk. This generalization lead to discussion of the usefulness of doing further human feeding tests. Dr. E.S. Anderson reported on the outline of experiments, that had been proposed to WHO and later COGENE, which would involve feeding of a standard set of* E. coli *and* E. coli *containing plasmids to laboratory worker volunteers in various laboratories throuhout the world. It was generally agreed that although no one was terribly concerned about the pathogenicity of* E. coli *K-12 or the increase in pathogenicity as a result of a cloning experiment such an experiment might help to strengthen the conclusion that* E. coli *K-12 is a very poor colonizer and would probably be so whether or not it contained a plasmid.*

The next topic that was discussed related to plasmids and estimates of their transmissibility with a goal of designing a possible test system to get further information on this question. Dr. Roy Curtiss presented evidence from in vitro *testing which gave the mobilization frequency for various plasmid components of EK2 vector systems. The data were taken from tri-parental matings which went on for about 60 minutes under conditions where the conjugative plasmid was already in the host into which the test plasmid was placed. Following is a table of these results:*

Plasmid	*Markers*	*M daltons*	*Mobilization Frequency*
pCR1	*ColE1,* Km^r	*8.6*	10^{-1}
pSC101	Tc^r	*6.0*	10^{-4}
pMB9	*ColE1,* Tc^r	*3.5*	10^{-7} — 10^{-8}
PBR313	*ColE1,* Ap^r*,* Tc^r	*∿6.7 (approx.)*	
pBR322	*ColE1,* $A_p{}'$*,* Tc^r	*2.6*	

Curtiss emphasized that these tests show the highest measurable frequencies. In the gut, the normal environment of these organisms, there are many factors, e.g. low pH, presence of bile, the long generation time of the cells, etc. all of which could be expected to inhibit conjugative transfer. He showed a table which assigned a probability factor for all these parameters, and then expressed the total probability (expressed as transfer per day) for the standard K-12 systems. This number comes out to be 10^{-16} *for pBR322 in wild type K-12 systems, and* 10^{-29} *when the host is* $_x$*1776. It seems clear from these data that transmissibility, at least with nonconjugative* plasmids, does not seem to be a parameter of major concern in recombinant DNA research. This conclusion was supported by the evidence of studies reported by Dr. S. Levy in which he did tests in which both farmyard chickens and the farm family which took care of them were exposed to plasmid-containing bacteria resistant to tetracycline. It was shown that when the tetracycline resistance marker was transferred between animals and from animals to man this transfer was not via the plasmid but colonization with bacterium itself. It is also noteworthy that the plasmid in this test was a conjugative plasmid. Curtiss also reported that over 160 E. coli strains isolated from Nature have now been tested for "nonsense" suppressor function and all have been found negative. His tests included organisms isolated from the gut, from water, and from bacteremic patients in hospitals. Thus, it would appear that amber mutations will afford safety.

Despite the evidence that transmissibility is probably not a matter of concern, and that in vitro tests probably give the results of the "worse possible case", it was still felt that perhaps some sort of in vivo test might be useful. This test would be designed not so much to ask whether transfer of conjugative plasmids would occur, but whether or not there is some other mechanism of transfer among organisms in the gut that we are not yet aware of. Accordingly, it was suggested that the most relevant test of this particular hypothesis would be to place a marker on a nonconjugative plasmid and then to put this plasmid into various strains of E. coli. After feeding them to test animals, (mice or even humans) one would ask whether transfer of any sort can be detected. The results of the test would be expressed as the frequency of transfer of this nontransferable, nonmobilizable plasmid over the frequency of a transfer positive, mobilizable positive plasmid.

Discussion next centered on assessment of the likelihood of production of an "effective" toxic substance by a cloned gene product either in the gut (the reservoir of most concern) or in the urinary tract. It was noted by Sidney Brenner that perhaps a good deal of information relative to this topic is already available. One could, for example, obtain a partial assessment by asking whether one could detect an immune response to various bacterial proteins which are contained inside of the cells or on the surface. Detection of immune response would, of course, be considered evidence that the bacterial proteins had been "seen" by the host defense mechanism. Examples of markers which were suggested were the pili surface proteins, beta galactosidase as an internal protein and perhaps something like penicillinase which is excreted by some bacteria due to the presence of a plasmid. These sorts of tests would be rather straightforward and involve only serum testing of a variety of laboratory animals as well as human beings.

Another topic which received a fair amount of discussion concerned ways to assess the effect of cloned fragments from shotgun experiments. A method of testing was proposed by Dr. David Bot-

stein, based on a model outlined by Dr. Ron Davis. This model involved tests of lambda carrying yeast shotgun fragments, serially passaged through coli. *The object of these tests was to arrive at some rational way of dealing with populations of clones which would include collectively, the entire gene content of a particular organism. It seemed most reasonable to start with yeast because its genome is much smaller than most eukaryotes. Further tests could include more complicated eukaryotes. The test would involve taking an entire shotgun from a cloning experiment and to passage it through animals (either normal or germ-free mice for example). After colonization was established, the bacteria would be reisolated, and checked to see whether any clones had survived better than others. The "winners", those present in highest number, would be assumed to be those which were best adapted for colonization. These could then be compared by various criteria with wild type* E. coli. *The results with cloning of yeast genes in* coli *on the lambda chromosomes have shown that in every case in which good survivors were selected and checked by comparison with wild-type lambda, they grew less well than the wild-type strain.*

The question then arose, what does one do with clones that have been purified from shotgun experiments? What sort of test could be required of such clones? The participants in the conference were unable to offer any concrete test in the particular situation other than using the same sort of tests that had been outlined for the shotgun. It is clear, however, that some way to cope with these kinds of clones must be developed, since most Guidelines place shotgun experiments in a higher risk category than those using purified genes simply to compensate for the lack of knowledge concerning possibly harmful gene elements within the population. Some way of putting a value on that of the "lack of knowledge" component has to be developed if guidelines are to evolve rationally.

Another topic of discussion was centered on experiments involving animal virus DNAs. Here the critical question was whether or not the cloning procedure would in any way create a path of entry for animal viruses that would bypass the normal defense mechanisms of the host. If so, then containment higher than that required for the animal virus itself would be warranted. However, it was considered equally possible that putting animal virus DNA into E. coli *would really represent an attenuation of viral virulence. If it turns out that attenuation is the rule, then cloning may represent a preferred way to work with animal virus pathogens. It was considered absolutely essential that risk assessment experiments to test the effects of cloning animal virus genes be undertaken. There was unanimous agreement that the Martin and Rowe type of experiment (that is, cloning of polyoma DNA in* E. coli *and exposure of mice to the recombinant clones in various ways) constitutues the most sensitive and least hazardous way to test this question. Drs. Martin and Rowe have in fact done some preliminary non-cloning experiments in anticipation that they would soon have the facilities to do these kinds of* experiments. At the moment, however, it seems that their ability to proceed is being bogged down with bureacratic problems. It is anticipated that once the results of this particular animal virus experiment were available, others equally interesting for example, with RNA tumor virus genes, other DNA tumor virus genes, etc. might be designed to test further implications of cloning animal virus genes.

An experiment related to the one above, in that it attempts to answer the reverse question, was proposed by Allan Campbell. The attached material contains an outline of the experiments that he proposed. Briefly, Campbell's experiments are directed at the question of whether genes contained in eukaryotic cells can be passed into coli. He suggests a quite rational approach to this which would involve use of a gene initially present in E. coli and therefore most capable of functioning in E. coli if it could return. One example given was the coli gene for galactose utilization. This could be placed in an animal virus vector, (polyoma or SV40) and then into an animal cell. The test would be to grow an E. coli strain deleted for the gal gene in the presence of this animal cell (either in tissue culture or after the cell had been put into an animal where it could develop a tumor) and ask whether the E. coli mutant could pick up the gal marker from the animal cell. This seemed to me an elegant and simple first attempt to test for the existence of the hypothetical barrier to genetic exchange between eukaryotics and prokaryotes.

In summary then, a number of specific risk assessment tests were proposed at this meeting. These include:

1. Colonization and transmission test of E. coli and E. coli-containing plasmids as summarized by Anderson and contained in material already available to COGENE.
2. Tests of shotgun clones by passage through animals and selection of "winners" as outlined by Botstein and Davis.
3. In vivo (feeding) tests of transmissibility by mechanisms other than the known conjugative functions.
4. The test of animal virus clones such as the Martin and Rowe polyoma test and
5. The reverse of this, that is the Allan Campbell test for flow of genetic information from eukaryotes to prokaryotes.

Also noteworthy was the unanimous agreement that it must be considered virtually impossible to convert E. coli K-12 into a pathogen of epidemic consequence by insertion of random bits of eukaryotic DNA.

Section V
COMMENTS ON OTHER RELEVANT ACTIONS AND REPORTS

(1) Report of a U.S. National Academy of Sciences Panel on Risks and Benefits of Recombinant DNA Research Performed under the NIH Guidelines (Nov. 1, 1977).

(2) Testimony of Philip Handler, President of the U.S. National Academy of Science, before a U.S. Senate Subcommittee Hearing (Nov. 2, 1977).

(3) Falmouth Workshop (June 20-21, 1977).

(4) International Symposium on Genetic Engineering, co-sponsored by WHO and the Lorenzini Foundation (March 29-31, 1978).

(5) Proposed revised NIH Guidelines (September 1977) and Public Meeting for Consideration (Dec. 15 and 16, 1977).

(6) Report of the Workshop to Assess Risks for Recombinant DNA Experiments Involving Viral Genomes, co-sponsored by NIH and EMBO (Jan. 26-28, 1977).

A number of reports have been compiled in connection with meetings and hearings which contain material relevant to risk assessment. Many of these are listed in the bibliography or referred to by the various respondents to our Questionnaire. These include:

(1) A report of the "Panel on Risks and Benefits of Recombinant DNA Research Performed under the NIH Guidelines" prepared by an ad hoc Committee of the U.S. National Academy of Sciences (Nov. 1, 1977); and

(2) Testimony of Philip Handler, President of the U.S. National Academy of Sciences before a U.S. Senate Subcommittee's Hearing on November 2, 1977.
(These documents are not readily available and are therefore included in their entirety in Part II of this report).

Members of the Panel mentioned above were chosen for their special competences and with regard for appropriate balance, from among members of the U.S. National Academy of Sciences, the National Academy of Engineering and the Institute of Medicine. This *ad hoc* panel was formed in response to a request from U.S. Congressman Paul Rogers; Chairman of the Subcommittee on Health Affairs of the House Committee on Interstate Commerce. Their report, which was approved by an independent group from the same societies, reviewed the history which led to the controversy surrounding Recombinant DNA Research and to the development of the NIH Guidelines. They considered potential risks and benefits, within the context of these Guidelines. The report lists six items considered to embody the major causes of concern regarding recombinant DNA research, and discusses the validity (or lack of it) for each one at great length. The six items are:

Could the use of *E. coli*, a known inhabitant of the human intestinal tract, as the host organism for recombinant DNA experimentation lead to epidemics?

Could recombinant DNA harbored in *E. coli* be transferred to other organisms in the intestinal tract?

Could novel organisms be created that could be ecologically disruptive or cause human or animal disease?

Are physical and biological barriers used in the experimentation adequate to prevent the escape of hazardous organisms from the laboratory?

Could genes from eukaryotic cells be transferred into prokaryotic cells and express their genetic information in ways that could be harmful?

And finally, is it possible to develop for use in experimentation an organism so fastidious in its growth requirements as to be unable to grow outside of the laboratory?

At the end, they concluded the following:

" . . . *In summary the Panel believes that the body of evidence acquired or adduced over the last few years clearly indicates that recombinant DNA research when performed under the NIH Guidelines presents no real risk to public health. Past experience teaches that no manifest epidemics have ever arisen from laboratory work even with extremely pathogenic and/or contagious organisms and recombinant DNA work uses non-pathogenic organisms with diminished communicability. Knowledge of the biology of EK-2 hosts and vectors and of the available physical containment facilities gives confidence that when the research is in the hands of trained workers no untoward events will occur.*

We conclude with the majority of scientists that many of the concerns first expressed by scientists in 1973, have been satisfactorily answered. Significantly, several scientists who had first called for extreme caution have now stated their belief that research under present guidelines is safe . . . "

They state further:

" . . . *The discussion of the concerns which have been raised regarding recombinant DNA technology indicates that they are largely without foundation. Indeed, it is probable that most recombinant DNA work using enfeebled* E. coli *K-12 systems could be carried out safely without any special precautions. Consequently, the public and its representatives in legislative and administrative offices should consider carefully the risks of overregulating this kind of endeavor.*

Recombinant DNA research under the NIH Guidelines has such great promise for rapid future benefits and so little chance of causing harm that its regulation should be implemented with a minimum of restriction. Cumbersome and punitive legislation is not needed. The financial cost of overly cautious containment and enforcement, the delay in achieving benefits, and the penalties incurred by restricting freedom of inquiry are real risks to be considered in setting up regulations . . . "

The closing statement of this report is an assertion of their belief in the present and future benefits to be derived from the application of those techniques.

" . . . *Finally, we state in summary and with assurance that the application of recombinant DNA techniques has already led and will lead to important increases in our understanding of human biology in both health and disease* . . . "

A copy of the Panel's report was submitted "for the record" by Dr. Philip Handler, President of the National Academy of Sciences during his testimony before the U.S. Senate Subcommittee on Science, Technology and Spaces. Dr. Handler's testimony addresses itself primarily to the possible effects of governmental regulation on freedom of inquiry. He states:

" . . . *The objective of some who have proposed the regulation of recombinant DNA research is to use the power of government for the suppression of ideas that may otherwise flow from such research. That would take us back to an era of dogmatism from which mankind has only recently escaped. And it would be a feckless task. In the long run, it is impossible to stand in the way of the exploration of truth. Someone will learn, somewhere, sometime* . . . "

and later:

" . . . *Understanding of the genetic process is the principal objective of such (Recombinant DNA) research. But the practical application to be explored is not to tamper with man's genetics, it is to repair them if and when that proves possible. In evidence, may I submit this, as yet incomplete, yet woefully long listing of the hereditary diseases of man, taken from the proofs of the next edition of the textbook of biochemistry of which I have been coauthor for 25 years. These are diseases for which one may earnestly seek therapy or prevention; and that is a principal form of "genetic engineering" to which we hope that research on recombinant DNA may one day contribute.*

The technique of recombination of DNA has already proved its power to illuminate for us the nature of the genetic apparatus which is the essence of life. And there is much, much more to be done. Accordingly, I beseech the power of the First Amendment in asking the Congress to abjure any legislation which, by accident or design, will limit the freedom of inquiry, save for express and compelling cause as elsewhere instructed by the Constitution . . . "

Although Handler agreed that until more evidence is in hand all research in this field should be conducted as specified by the NIH Guidelines, his final remarks include a note of warning regarding risks associated with legislative enforcement:

" . . . *Mr. Chairman, you who consider such legislation will appreciate that you have embarked on a thorny trail. The risk of violating that which we cherish under the First Amendment surely looms as large as the hypothetical risks against which these safeguards are being erected. And I am concerned that each instance of regulation of research will facilitate the next. Do be very careful. An excess of zeal to protect us against all risks, however minor, particularly when seen* *against the backdrop of our chancy world, could seriously cripple science, the principal tool our civilization has fashioned to mitigate the condition of man . . . "*

The above and many other similar documents refer to:

(3) The NIH-Sponsored "Falmouth Workshop" of June 20-21, 1977 (see Section IV).

The proceedings of this meeting are expected to be published in the May 1978 issue of The Journal of Infectious Diseases.

Additional relevant information was presented at:

(4) The Lorenzini-WHO-Sponsored "Symposium on Genetic Engineering" (March 29-31, 1978).

This meeting was attended by all three members of our Working Group. The Proceedings will be published by Elsevier-North Holland in late 1978. Among the many interesting scientific presentations, participants learned of still another group's success in detecting expression of eukaryotic genes (*Neurospora;* S.R. Kushner et al., Univ. of Georgia, USA) in *E. coli* bacteria and the perhaps surprising lack of successs on the part of two other groups in attempts to detect expression of *E. coli* genes introduced into another prokaryote, *B. subtilis* (W. Goebel, el al., Univ. of Würzburg, Germany; S.D. Ehrlich et al., Univ. of Paris, France). This prompted J. Tooze (EMBO) and others to question the logic of many guidelines in which the level of containment is determined on the basis of phylogenetic relatedness.

The development of an efficient transformation system for yeast was another report of great interest. Dr. J. Fink (Cornell Univ., USA) presented evidence that DNA can be readily taken up by these organisms, and an individual segment can be inserted in many locations in the yeast genome. Bacterial plasmid DNA is inserted along with the yeast genes, a finding which challenges the existence of the hypothetical barrier to DNA exchange between prokaryotes and eukaryotes. Dr. A Rörsch (University of Leiden, Netherlands) summarized results from his and other groups on the transfer of plasmid DNA from the bacterium *Agrobacterium tumefaciens* to plants, a second example of prokaryotic-eukaryotic DNA exchange. He discussed ways in which the plasmid may be used as a recombinant DNA vector in plant cells.

In another series of reports, participants learned from the groups of Tonegawa (Basel Institute, Switzerland) Chambon (CNRS, France) and Kourilsky (Pasteur Institute, France) that eukaryotic genes for differentiated function (immunoglobin and ovalbumin) are interrupted by many non-gene spacer regions which are later removed during formation of mature messenger RNA. As far as is known, prokaryotes possess no analogous mechanism for RNA processing. Thus, it seems probable that many genomic eukaryotic DNA sequences may not be expressed in *E. coli* or in other prokaryotic hosts.

Dr. H. Boyer (U. of Calif., USA) summarized his and his colleagues work (see bibliography) leading to the synthesis and cloning of a "synthetic" gene for the brain hormone somatostatin. The hormone is safely synthesized in bacteria as a non-functional protein and can be released later in a contained *in vitro* chemical reaction.

In another session of this symposium, participants heard from several members of industry (J. Boldingh, Unilever Research, Netherlands; I.S. Johnson, Eli Lilley, USA: and M. Soria, of Carlo Earba, Italy), all of whom expressed strong support of the aims and intent of various guidelines and "codes of practice" governing recombinant DNA research. Although industrial involvement at present is minimal all expressed a confidence that the recombinant DNA techniques would some day result in significant benefits to medicine and agriculture.

(5) Proposed revisions to the NIH Guidelines.

The proposed revisions were published in the U.S. Federal Register in September 1977 and discussed during a two day public meeting at NIH, December 15 and 16, 1977.

Summary minutes of this meeting and a detailed justification for the revisions prepared by the Recombinant DNA Molecule Program Advisory Committee, have been published in a supplement to the Fall 1977 issue (Vol. 1, No. 1) of the Recombinant DNA Technical Bulletin. This supplement is available through the NIH Office of Recombinant DNA Activities, Building 31, Room 4A52, NIH, Bethesda, Md. 20014, USA.

The introduction to the proposed Guidelines states that "the present revisions take into account many communications from both scientists and non-scientists since the original publication of the Guidelines". With respect to risk-assessment it goes on to state that:

> *"During this period the committee has also become better informed about the general ecology and epidemiology of infectious microorganisms. Of particular relevance has been the information received from many medical microbiologists, including data from experiments with* Escherichia coli *K-12. These experiments include a demonstration that strain K-12 cannot be made pathogenic even when provided, by standard genetic techniques, with the genes for known toxins and other pathogenic properties. Other relevant experiments that have been reported show that the incorporation of foreign DNA does not increase, but rather tends to decrease, the general fitness of microorganisms; this phenomenon further contributes to the unlikelihood that cells carrying recombinant DNA will survive in Nature. Indeed, everything we have learned tends to diminish our estimate of the risks associated with recombinant DNA in* E. coli *K-12. Nevertheless, the revised Guidelines continue to be deliberately restrictive, with the intent of erring on the side of caution."*

A list of the sources by which the committee has become "informed" is contained in Part B of the Technical Bulletin mentioned above. It includes material presented at the Falmouth Meeting (See Section IV of this report) as well as other published data. Much of this material is not new information, but data familiar to experts in epidemiology and medical microbiology. Thus, it is clear that a larger body of information relevant to risk assessment is already available. It is significant that familiarity with this information has tended to diminish estimates of risks.

Three members of COGENE, Drs. J. Tooze, W.J. Whelan and A.M. Skalka, were present at the December public meeting. Following is a short report of the proceedings by A.M. Skalka:

Special Meeting of the Advisory Committee to the Director, NIH, to Discuss Draft Proposed Revised Guidelines for Recombinant DNA Research

On December 15-16, 1977, I attended this open meeting which took place at the NIH in Bethesda.

The meeting was attended by many spectators including members of the press, members of Congress, the NIH Recombinant DNA Molecule Advisory Committee (RAC), foreign visitors and scientists from the NIH. Dr. Frederickson presided and the text of his opening remarks was available. In addition, to the permanent members of the Advisory Committee to the Director, many ad hoc members were added for this meeting in order to insure as diverse a representation of background and opinion as possible. Thus, the membership included lawyers, a representative from the Natural Resources Defense Council, a professor of theological ethics, student and technician members of two local Biohazard Committees as well as scientists from many disciplines. The invited witnesses were also chosen to represent a broad spectrum. Not listed but present and quite vocal was Ms. Francine Simring of "Friends of the Earth". In addition, several public witnesses not listed also spoke. Among these were: Dr. James Watson of the Cold Spring Harbor Laboratory, Dr. Jonathan King of MIT, Dr. Waclaw Szybalski of the University of Wisconsin, Dr. John Tocze of the European Molecular Biology Organization, and myself. A great deal of material was provided to invited guests and participants for their review before the meeting. The information included the "NIH Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules", a "Report of the Federal Interagency Committee on Recombinant DNA Research: International Activities", a pamphlet entitled, "Background on the Proposed Revisions (9/27/77) of the NIH Guidelines for Research Involving Recombinant DNA Molecules" as well as two booklets containing a collection of letters received by Dr. Fredrickson with comments on the proposed revised Guidelines.

The various sections of the proposed revised Guidelines were discussed in turn. After a general discussion of the changes and the reasons for them, there were five-minute presentations by invited witnesses. Questions on the "Introduction" focused primarily on the new definition. Several changes were suggested to make the definition more precise. Comments from invited and public witnessess followed expected lines. Representatives of the "Sierra Club", "Friends of the Earth" and the "Environmental Defense Fund" took this opportunity to voice their opposition to any loosening of the Guidelines and to call for enactment of federal legislation. Comments from others, including scientists from the United States and abroad, stressed the necessity for revision to keep the Guidelines abreast of new knowledge.

In the next section, Dr. Emmett Barkley from the NIH discussed changes in the "Physical Containment" requirements. He explained that many of these changes had come about after a joint meeting sponsored by NIH and the European Molecular Biological Organization in which physical containment procedures were discussed in depth and an attempt was made to harmonize containment procedures used in foreign countries and the United States. There was not much discussion in this section except for Dr. Barkley's explanation (after queries) of (1) why it is unnecessary to filter air exhausting from P3 laboratories (with the exception of that coming from biological containment cabinets) and (2) why it is unnecessary to require that every P3 laboratory have an autoclave within the restricted area.

In the next segment of the meeting, Dr. Susan Gottesman explained the changes in the "Biological Containment" section. The major innovation in this section is the introduction of the HV terminology (host-virus). This was done in order to allow for the development of host vector systems in addition to that utilizing E. coli K-12. The RAC considered that this was an important and necessary change. The requirements that all new HV systems be certified by the RAC will insure that there will be a constant surveillance of the types of systems to be used.

Some of the most heated and important discussions of the entire meeting took place in the discussion in the next two sections; changes in the "Experimental Guidelines" and "Roles and Responsibilities". It became apparent after presentations by Dr. John Tooze of the European Molecular Biology Organization and various representatives from the plant virus fields that the Experimental Guidelines outlined for animal virus studies and for plant and plant virus studies are still unnecessarily restrictive. John Tooze made the statement that in the opinion of many European animal virologists, and of those in EMBO, the NIH Guidelines for animal viruses were strongly influenced by what he considered "political" considerations. Several members of the Advisory Committee expressed consternation at this fact. They believed that the Guidelines should reflect a true scientific evaluation rather than political considerations. I believe that Dr. Fredrickson is considering sponsoring a meeting of European and American animal virologists in the near future in order to rectify this situation. It is hoped that this meeting can come up with recommendations which will be consistent with the best scientific evaluations of possible hazard. An important bit of information that was revealed at the meeting and had significant impact was the inability of the animal virus groups in the United States to perform any animal virus risk assessment experiments. This ability arises from the severe restrictions placed on these experiments by the Guidelines. John Tooze announced that the same risk assessment experiments that have been planned for at least 2 years in the United States (but not yet initiated) are now being carried out in Europe. It was the general agreement that the problems with the "Experimental Guidelines" relative to plants and plant viruses stem from the fact that these fields are not adequately represented in the RAC and attempts will be made to rectify this situation as well.

Discussion on the section of "Roles and Responsibilities" was also very lively. Many suggestions were made to specify more clearly the duties of local Biohazards Committees. Some question was brought up by the environmentalist groups whether these Biohazards Committees could, in fact be relied upon to perform their duties seriously and conscientiously. Local Biohazards Committees were supported most vigorously by Mr. Dennis Helms, Special Assistant to the New Jersey Attorney General and an ad hoc *member of the Advisory Committee, who assured all present that his experience with the various Biohazards Committees in the State of N.J., indicated that these Committees did take their responsibilities quite seriously and in his opinion were capable of executing those responsibilities conscientiously.*

Nicholas Wade's article in Science (Jan. 6), reports on some of the more "glamorous" comments made during the session in which each member of the Advisory Committee gave a short oral summary of his impressions (fuller, written summaries will be mailed to Dr. Fredrickson). What Wade's article fails to stress, is the strong feeling expressed by a majority of the members of this Committee, that the present proposed revisions certainly seem justified and should be adopted. Several members expressed the view that even further relaxation seemed justified in several areas (i.e. animal virus and plant "Experimental Guidelines").

Before adjournment, Dr. Frederickson announced that the records would be left open for another 30 days and he invited comments from all interested parties. Based on these, and comments that he will receive in writing from members of his Advisory Committee, he will decide whether to accept the proposed revised Guidelines on Recombinant DNA Research.

(6) U.S.-EMBO Workshop to Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses, Jan. 26-28, 1978.

As was suggested in the above report on the December 15-16 meeting at NIH, this Workshop was held in response to discussion concerning viruses. The Virus Workshop, like that at Falmouth, was a meeting of experts in the discipline of interest. The meeting held in Ascot, England was attended by 27 scientists from the U.S., W. Germany, Finland, France, Sweden, and Switzerland. One member of COGENE (J. Tooze) was among the participants. The purpose of the meeting was "*to conduct a scientific and technical analysis of possible risks associated with cloning eukaryotic viral DNA segments in* E. coli *K-12 host-vector systems and with the use of eukaryotic viruses as cloning vectors in animal, plant, and insect systems*". A full report appears in the U.S. Federal Register of March 31, 1978. With respect to cloning in *E. coli* K-12, the group concluded "*That the probability that K-12 organisms carrying viral DNA inserts could represent a significant hazard to the community was so small as to be of no practical consequence*". A lengthy discussion outlines the evidence supporting this conclusion and the strong feeling that "*viral genomes or fragments thereof, cloned in* E. coli *K-12 using approved plasmid or phage vectors pose no more* *risk than work with the infectious virus or its nucleic acid and in most, if not all cases, clearly presents less risks".*

Based on included considerations, the report ends with specific recommendations for containment. It recommends use of P2 (NIH Guidelines) or CI (William's Report) containment measures, in conjunction with an EK1 host vector system for cloning any viral genome or fragment thereof. In cases where the virus itself must be handled at higher levels of physical containment, they suggest use of the more stringent condition. The group endorsed certain portions of the NIH Guidelines dealing with animal virus vectors and recommended changes in others. A table is included to summarize the recommended containment levels for various viral vector and foreign DNA combinations which range from L (Low or P2), M (Moderate or P3) to CbC (for "Case by Case") determination. Conditions under which vertebrate host-vector systems might be used are also suggested. Finally, use of baculoviruses and cauliflower mosaic viruses as vectors are also considered.

Section VI
COGENE-INITIATED RISK ASSESSMENT EXPERIMENTS:

(1) Recommendations from March 5, 1977
Richmond-Falkow-Anderson Meeting
(2) Recommendations from March 30, 1978
Richmond-Falkow-Anderson Meeting
(3) Recommendations of the Working Group
on Risk-Assessment. — April 3, 1978

OUTLINE PROTOCOL OF TESTS FOR COLONIZATION AND TRANSMISSION OF *E. COLI* AND *E. COLI*-CONTAINING PLASMIDS

The following outline was submitted to COGENE by Professor E.S. Anderson, S. Falkow and M.H. Richmond . . .

"Even though a certain amount of work on the survival of E. coli *K-12 in man has already been published, we feel that there is a case for carrying out a wider study. In view of the particular concern of COGENE, we feel that experiments should be of two broad types. First, studies in which volunteers swallow marked* E. coli *lines carrying appropriate bacterial plasmids and the excretion of the strains and of the plasmids is then determined; and secondly, studies in which workers involved in "genetic engineering" are monitored to see whether they ever excrete detectable numbers of the bacteria and plasmids with which they are working.*

As far as the feeding experiments are concerned, we would broadly envisage two types of experiment. In the first, a nalidixic acid resistant E. coli *K-12 line, carrying a well defined self-transmissible plasmid with an unusual marker pattern, such as trimethoprin + kanamycin resistance, would be used, and the excretion of other* E. coli *strains which had picked up other plasmids would be monitored. The object of this experiment, therefore, is to test:*

a) the survival of a fed E. coli *K-12 line,*

b) the frequency of transfer of a self-transmissible plasmid from that strain to the resident Gram-negative flora,

c) the frequency of transfer of other R-plasmids to the fed strain.

It was envisaged that about 20 volunteers would be used in each test centre, and it was thought an advantage if the strains were fed in milk to some of the volunteers, and in nutrient broth to others. The first procedure would be likely to give a "worst case" response, while the latter would be more characteristic of a laboratory accident.

The second type of feeding experiment would be broadly of the same type as the first, except that the E. coli *K-12 strain would be one carrying a non-self-transmissible plasmid with appropriate markers for* *recognition purposes. The object of this test would be to assess the probability of plasmid mobilization rather than self-specified transfer.*

We feel that the feeding experiments should be carried out in a limited number of laboratories (probably not more than 10-12), chosen to achieve two main objectives; first, there should be a number of laboratories distributed throughout the world in such a way that one might assess the impact of dietary factors on excretion and transfer. Such labs might be expected to be predominantly in the developing world. The second group of laboratories should be representative of those in developed countries where substantial amounts of genetic engineering are already in progress.

The monitoring experiments — as opposed to those concerned with feeding — would be confined to laboratories already carrying out genetic engineering experiments. Their numbers might well be more extensive than in those doing the feeding experiments. In this case, the test would amount only to plating faecal samples from the workers on appropriate agar to detect the strains and plasmids used for experimental work. For this, COGENE would have to encourage the labs concerned to mark their host strains in such a way that they could be recognized easily; and it seems possible that COGENE might give some advice as to how this might be done.

We gave some thought to the possibility of trying to set up experiments in which the excretion of vector plasmids might be followed in those doing genetic engineering. However, we find it hard to propose anything that would be likely to be accepted willingly in the labs concerned. The use of tetracycline resistance in vectors already causes difficulties for monitoring because of the prevalence of tetracycline resistant E. coli *in man at the present time; and we suspect that the use of antibiotic resistance markers in vectors is likely to be abandoned gradually in favour of other markers with less undesirable properties anyway. In view of this, we would not want to encourage the insertion of antibiotic resistance markers in vectors merely for the monitoring purposes. Perhaps the best plan in this case would be to watch developments and make proposals when the selective markers finally adopted for use in vectors have become clearer.*

There are two general (unconnected) points that are worth making. First, we do not feel that it is COGENE's role to validate hosts and vectors: that must be the responsibility of national committees. Secondly, we have made no proposals about setting up experiments with cloned eukaryotic DNA at this stage. Perhaps these could come later.

Finally, we think that a successful organization and completion of these tests will need a small steering group to oversee them. This group could also — in due course — be responsible for organizing the publication of the results of the tests. Unless this is done, the whole exercise will have been pointless".

March 1977

Recommendations from March 30, 1978 Richmond-Falkow-Anderson Meeting

To The Working Group on Risk Assessment

Discussion held March 30, 1978
E.S. Anderson, M.H. Richmond, S. Falkow

It was our concensus that we now have available sufficient data in man and livestock to show:

A) the survival of fed **E. coli** *K-12 sublines*
B) the frequency of transfer of a self-transmissible plasmid from K-12 to resident gram-negative flora
C) the frequency of transfer of other R plasmids to the fed strain, and
D) the frequency of mobilization of non-conjugative plasmids from K-12 to other enteric flora.

The combined monitoring data of our three laboratories takes into account well over three years of combined effort. The monitoring data encompassed:

1) research workers
2) their families, and
3) the laboratory environment.

We are prepared to offer concrete recommendations for monitoring the laboratory environment and laboratory personnel.

It is our recommendation that upon my arrival in Bristol in June 1978, I set out to correlate and analyze the data from our laboratories into a single publication. We would submit our results to COGENE by September 1978 and should be glad to learn whether it is felt further experimentation is necessary.

. . . Our major feeling is that subsequent to our report to COGENE of one year ago, we have accumulated sufficient additional data so that it is no longer necessary to undertake the wide study that was initially envisioned.

S. FALKOW
March 31, 1978

RECOMMENDATIONS FROM THE WORKING GROUP TO COGENE

Although it is clear from the comments of these experts on infectious diseases and plasmid ecology that many data exist which bear on risk assessment, much of the information is still of an informal nature, having been collected as a by-product of other efforts. The Risk Assessment Group welcomes the opportunity to support efforts to collect these data in a form which can be published in a scientific journal where they will be accessible to other scientific experts and laymen. Organization of these data may well reveal one or two points which should still be subjected to experimentation, in which case support should be given to allow the work to be undertaken without delay.

The Working Group also welcomes the recommendations of these experts regarding laboratory and personnel surveillance. A simple set of instructions would be most helpful to scientists not familiar with the routines of medical microbiology. Such monitoring might be used during training exercises or in working situations where appropriate.

We believe that the addition of these reports and recommendations to our final Working Group Report will be an important contribution to the scientific community and to others concerned with Recombinant DNA research.

Section VII

BIBLIOGRAPHY AND SOURCES

This section is not intended to be comprehensive. Additional references are included with many of the reports and compilations listed.

Reports and Compilations:

(1) Proposed Revised Guidelines; Recombinant DNA, Federal Register Vol. 42 No. 187 — Tuesday, Sept. 27, 1977.
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(2) U.S.-EMBO Workshop. To Assess Risks for Recombinant DNA Experiments Involving the Genomes of Animal, Plant, and Insect Viruses. Federal Register Vol. 43, No. 63. Friday, March 31, 1977. Available from the NIH Office of Recombinant DNA Activities (see (1) for address).

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Vol. 1, No. 1 includes the following articles of general interest:
"Current Status of GK lambda EK2 Vectors"; by A.M. Skalka (Phage Working Group)
"Certification of Host Vector Systems"; by The Plasmid Working Group
"Report of the NIH/EMBO Workshop Parameters of Physical Containment"; by E. Barkley & J. Tooze
"Report of Falmouth Workshop"; by S. Gorbach
"International Activities in Recombinant DNA Research"; by D. Kamely and R.B. Curtin
Vol. 1, No. 1 Supplement includes:
"Summary Minutes of Director's Advisory Committee Meeting, Dec. 15-16, 1977."
"Comparison of Original and Proposed Revised NIH Guidelines Justification for Proposed Revisions"; by Recombinant DNA Molecule Advisory Committee

(5) Science Policy Implications of DNA Recombinant Molecule Research.
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SECOND REPORT OF THE COGENE WORKING GROUP ON RISK ASSESSMENT

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COGENE's Working Group on Risk Assessment was established in May 1977 in response to concerns regarding the safety of recombinant DNA technology. Its members include: Drs. Giorgio Bernardi (France), Vittorio Sgaramella (Italy), and more recently, Dr. Natalie Teich (U.K.) with myself as convener. At its formation, the Group outlined two objectives. One was to gather information to be considered in the assessment of risks. The second was to identify possible areas from which additional relevant information might be obtained, and, if studies in such areas were not already underway, to consider their sponsorship.

In July of 1978, COGENE made public the first report from this Working Group[1]. This two part, 319 page report contains a variety of information including responses to a questionnaire sent to various international organizations and evaluation of their significance, reports of various meetings and hearings as well as comments on relevant information from the literature and opinions of individual scientists.

Analysis of this information indicated that the concerns regarding recombinant DNA research could be grouped into three major categories:

1. Organisms carrying recombinant DNA may spread in the natural environment and disrupt existing ecological equilibria.

2. These organisms might produce some toxic or noxious substance, or otherwise cause disease.

3. By exploiting this technology, scientists may be crossing some hypothetical barrier to DNA exchange between eukaryotes and prokaryotes and thus affect pathogenicity or dispersion of pathological agents.

Reports from the Falmouth Meeting (June, 1977) and COGENE-sponsored analyses of E. coli K-12 systems, both discussed in the report, showed that many relevant experiments had already been conducted by scientists in such closely related fields as epidemiology and infectious diseases. Their observations that E. coli

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[1] Copies of the report are available from COGENE (c/o Dr. W. J. Whelan) P.O. Box 016129, Miami, Fla. 33101, U.S.A.

K-12 has a limited potential of survival and their consensus that this bacterium cannot be converted into an epidemic pathogen by laboratory manipulations with DNA inserts, has done much to satisfy the first concern mentioned above. As recently reported in the literature, (Petrocheilou and Richmond, 1977) results from long-term monitoring of laboratory workers who routinely handled K-12 organisms carrying transmission-proficient plasmids showed no bowel colonization despite the fact that the work was carried out without any special precautions. If the organisms which carry recombinant DNA cannot spread in the natural environment, then clearly the other concerns must also be diminished. The conclusion of a NIH/EMBO Animal Virus Workshop (in Ascot, Jan. 1978) was that recombinant organisms carrying viral inserts cannot be any more hazardous than the viruses themselves, and in some instances may provide an opportunity to work more safely with virulent agents.

As indicated in our report and in the proceedings of this meeting, important information on the nature and possible consequences of the manipulations used in recombinant DNA technology comes from experiments in a variety of fields. For example, attempts to understand the biological role of restriction enzymes have revealed that prokaryotic and eukaryotic DNAs can recombine in vivo. Other studies, including those with Agrobacterium tumefaciens and plant cells, also show that in some cases there is no barrier to exchange and expression of genes across the hypothetical prokaryotic-eukaryotic barrier. On the other hand, studies of animal virus genomes and eukaryotic genes for differentiated functions have revealed previously unsuspected possible barriers to their expression in prokaryotic backgrounds. It is clear that further studies of the molecular genetics, pathogenicity, ecology and other properties of organisms will continue to provide information relevant to the assessment of risk and that this source is no less important than specifically targeted experiments.

Replies to our Questionnaire indicated that there were several projects underway directed specifically towards risk assessment. These included the two separate polyoma virus experiments sponsored by EMBO and by NIH and several NIH contracts aimed at testing and verification of EK2 and EK3 systems. Preliminary results, as well as conclusions from the NIH-EMBO Virology Workshop indicated that these studies were unlikely to reveal unknown hazards. However, these and other experiments aimed at elucidating various aspects of the ecology and natural history of microorganisms should provide useful information and will be important in the development of host-vector systems other than those based on E. coli K-12.

In summary, as of July 1978 our analyses had revealed no scientific findings to justify any of the three concerns listed above; no risk unique to recombinant DNA research was identified. Available evidence indicated that recombinations of the type made possible by this new technology could occur in Nature. Evaluation of E. coli K-12 showed that this bacterium is essentially harmless and that insertions of segments of foreign DNA into its genome could not alter this property. With few exceptions, it seemed likely that the same would prove true of other host bacteria.

Some of the results discussed in our report were also documented in material which accompanied publication of the December 1978 version of the U.S. NIH Guidelines. This material was included as part of the justification for relaxation in containment requirements for permissible experiments.

With respect to the second objective of our Working Group, at the time of our first report it appeared that additional information on results from feeding experiments and monitoring for acquistion of laboratory E. coli strains would be useful. In these efforts we were fortunate in being able to enlist the aid of three distinguished investigators, Drs. S. Falkow (Seattle), Dr. M. Richmond

(Bristol) and Dr. E. S. Anderson (London) who had been conducting routine monitoring of their personnel involved in bacterial and plasmid research. These three scientists agreed to collate the results of their surveys for COGENE. Their conclusions, which were summarized by Dr. M. Richmond during this meeting, are included as Appendix II of this report. In our view these findings are of special importance because they record experience from real working situations in which no more than accepted microbiological practice has been employed. The results showed that under these conditions no acquisition of bacteria or even transmissable plasmids could be detected.

Additional data which bear on the assessment of risks has become available since our first report. These include results from the "polyoma" experiments now documented in the literature (Israel and co-workers, 1979; Chan and co-workers, 1979) and in preceding contributions in this volume. These studies, meant to be "worst case" tests using an extremely sensitive assay, show that polyoma-containing recombinant hybrids or hybrid-containing bacteria are either non-infectious or are less infectious in mouse than the virus itself. Thus, they constitute strong support for the conclusions from the Ascot Animal Virus Workshop mentioned above.

Finally, as mentioned above, specifically targeted projects designed to provide information on the survival potential of recombinants or recombinant-containing E. coli in aerosols, sewage, and the intestines of mouse and man have been the subject of several contracts supported by the U.S. NIH. Although these studies are still not complete, progress reviewed at a March 19, 1979 meeting at NIH also tendsto confirm original estimates on the relative enfeeblement of those systems. Appendix I of this report is a summary of that meeting prepared by Dr. J. Setlow, Chairman of the NIH Recombinant Advisory Committee, who presided.

How do these findings effect the future goals of our Working Group and what advice can COGENE offer its' sponsoring scientific unions and national organizations at this time? We conclude, as in our first report that no special risk inherent to recombinant DNA research has been defined. In the one case most thoroughly studied, polyoma virus, the recombinants clearly present less risk than the virus itself. With respect to experiments involving E. coli K-12 systems, it appears that little if any grounds for concern remain. Regardless of the nature of the cloned fragment or even its ability to be expressed, accepted microbiological practice should be sufficient to prevent escape and survival. These conclusions are especially reassuring because they serve to restore confidence in the ability to predict the outcome of recombinant DNA activities based on available knowledge of the biology of the organisms involved. Similar facts relating to other potential cloning systems should prove equally predictive and fewer verification experiments should be required.

However, despite our confidence in the relative safety of recombinant DNA techniques as they are currently used in basic research, our Working Group does not consider it justified to extrapolate from experience with E. coli to all possible experiments in the future. As technology related to genetic experimentation evolves, there are sure to be operations and applications that we can not even envision at this time. Thus, it is important that a Committee of scientists international in scope, continue to follow developments which relate to genetic experimentation, that it continue to collect information to be made broadly available and that it stand ready to sponsor specific projects which may be necessary for further evaluation.

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APPENDIX I

REPORT ON THE PHAGE AND PLASMID SUBCOMMITTEE ON MEETING WITH RECOMBINANT DNA CONTRACTORS

March 19th, 1979 in Bethesda

Jane K. Setlow, Chairman

SUMMARY OF REPORTS FROM CONTRACTORS

Testing of Host-Vector Systems in Sewage. (Bernard Sagik, James Walker and Charles Sorber, University of Texas at San Antonio)

The testing was based on a model system set up in a laboratory, capable of taking 60 liters/day of waste. San Antonio waste was brought into the laboratory to be put into the system. Data were presented to show that the various parts of the model system behaved quantitatively like those of real sewage processing systems (with respect to such parameters as detention time, pH, temperature, removal of solids, etc). The organisms to be tested (χ1776 and DP50*supF* and Charon 4A) were added continuously for 48 hours. Samples were taken at intervals up to 120 hours from nine parts of the treatment plant. For assays of DP50*supF*, the antibiotics nalidixic acid and trimethoprim were used, which kept the indigenous bacterial population from entering appreciably into the counts. These plus cycloserine were also used for the assay of χ1776. The Charon 4A phage was distinguished from the indigenous phage by plating it on XG agar, such that the plaques of these phage had blue-green halos. Considerable effort was put into verifying that the colonies obtained from the putative χ1776 that had passed through the treatments were really χ1776. Seventy-five colonies were tested for other markers such as phage susceptibility, efficiency of plating in the presence of bile salts, and various nutritional markers.

DP50*supF* to some extent multiplied during passage through the first parts of the treatment plant, although it would not be able to compete with wild type *E. coli*. There was little or no multiplication of χ1776. The titer of DP50*supF* dropped from a high of around 10^7/ml down to around 10^2 in the last effluent during the time of the experiment, and was somewhat lower for the treated sludge. Comparable values for χ1776 were about an order of magnitude lower. Charon 4A phage titers dropped about three orders of magnitude.

Testing of Host-Vector Systems in Mammals (Rolf Freter, University of Michigan)

Cells (1-5 x 10^9) were placed in the stomachs of mice, and at various intervals the stomach, small and large intestines were assayed for the presence of colony-forming units of the test microorganisms. Feces were also assayed. A non-absorbable radioactive tracer and spores of thermophilic *Bacillus subtilis* were added at the same time as the cells to monitor the passage of material through the digestive tract. Five types of *E. coli* were tested: DP50*supF*, χ1776, a wild strain derived from mouse intestine, a wild-type human strain C25, and another wild type, χ1666. The results showed that whereas the wild strains could multiply in the gut, especially when passage time was slowed by morphine, DP50*supF* and χ1776 were killed in the gut, particularly when passage time was increased. The amount of cells recovered in the feces was less than one per cent of the original inoculum of χ1776, and up to 10% of the DP50*supF*. Germ-free mice were used in some experiments, which showed that even in such hosts χ1776 could not become a resident in the intestine unless it was a revertant no longer sensitive to bile salts, but even then it did not readily establish itself. DP50*supF* similarly only became implanted when it had lost its bile sensitivity.

Testing of Host-Vector Systems in Mice and Humans (Stuart Levy and Bonnie Marshall, Tufts University)

Two strains of *E. coli* were tested χ1776 and χ2236 (the latter χ1776 plus the plasmid pBR322). The mouse results were similar to those of the Freter study, in that no colonization was observed, even χ2236 in germ-free mice. No survival of χ2236 was detected after 24 hours in either germ-free or conventional mice.

The human study involved groups of four individuals who were kept free of other human contacts for the ten days of the experiment, in a room previously prepared for bone marrow transplants. In addition to assay of stool samples, hand, throat and nose swabs were tested for the presence of the microorganism previously ingested in milk (around 10^9 cells). No recovery of the test organisms was detected from skin, and there was recovery from throat and nose swabs only the first day after ingestion. In the case where three of the individuals were given χ1776 but not the fourth, no transfer was observed to the fourth. After 4 days χ2236 survived only to the extent of 5×10^{-6} in its passage through the digestive tract, and χ1776 somewhat less. No recoveries were detected after 4-5 days. It was not clear whether the plasmid pBR322 really slightly increased the survival of χ1776 in the human gut (nor was it certain that the plasmid-containing χ2236 had no properties different from those of χ1776 in addition to the presence of the plasmid). Two hundred isolated colonies obtained from stools 4 days after ingestion were tested by the contractor and also colonies obtained from stools 4 days after ingestion were tested by the contractor and also colonies were sent to Roy Clowes and John Donch for further checking that the cells indeed had the characteristics of the test organisms. The presumed χ2236 isolates contained pBR322 and no other plasmid by the criterion of restriction analysis, and in no case was there detectable mobilizability of the plasmid. In all other respects (antibiotic resistances, nutritional requirements) the isolates had the expected properties.

Testing of Host-Vector Systems in Situations Simulating Accidental Spills (Melvin Hatch and Mark Chatigny, Naval Biosciences Laboratory)

Survival of χ1776, DP50 *supF*, wild type χ1666, an *E. coli* strain 980 isolated from an infant with diarrhea and the EK2 Charon 4A phage was tested at various relative humidities and temperatures on various types of laboratory surfaces, and also in aerosols. The latter measurements were made by obtaining the ratio of survival of the test organisms to that of *B. subtilis* spores resistant to aerosolization. As expected, χ1776 survives better under conditions not permitting any attempts at growth in the absence of diaminopimelic (dap) acid and thymine or thymidine (which could result in dap-less or thymineless death). The aerosol survival of χ1776 is only slightly lower than that of strain 980 at 50% relative humidity and 22°C, but much lower than that of χ1666. The two EK2 hosts, χ1776 and DP50*supF*, survived considerably less well on laboratory surfaces than did the wild type χ1666. Charon 4A phages were even more readily killed under comparable conditions.

CONCLUSIONS OF THE PHAGE AND PLASMID SUBCOMMITTEE CONCERNING THE CONTRACTORS' REPORTS

1) The data show no gross discrepancy between natural environmental decay of the EK2 microorganisms and the *in vitro* testing performed earlier (in which it was required that "no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under specified nonpermissive laboratory conditions").

The natural environment is actually a succession of individual environments, and when the loss of survival each of these is taken into account and multiplied together, the 10^{-8} figure is clearly obtained.

2) The original emphasis for the testing contracts was concerned with the possibility of converting EK2 systems to EK3. Since the revised Guidelines do not specify EK3 containment in any experiments, it was concluded that what was needed was information on survival of cloned fragments in EK1 experiments (such as in χ1666). Such experiments would, of course, be for risk assessment rather than in preparation for certification of vectors.

APPENDIX II

SUMMARY OF CONCLUSIONS FROM FEEDING AND MONITORING EXPERIMENTS[2]

M.H. Richmond, Bristol, UK

1. *E. coli* K-12, even prototrophs, fed to man and livestock in high doses will generally survive about 4 days, although in a few individuals the organism can be detected by selective methods for nearly 2 weeks. The organism may multiply to a limited extent within 24-28 hours after administration but generally does not undergo more than a few doublings.

2. If the *E. coli* K-12 that are fed carry a self-transferring plasmid that is de-repressed, the plasmid will be transmitted to the recipient flora. Transconjugants are found at a frequency of about 1×10^{-10} of all *E. coli* shed per day. No evidence for the mobilization of non-transferable plasmids from *E. coli* K-12 has been found for naturally occurring F group or I group R plasmids. We think it unlikely that such transfer would occur with approved cloning vectors. This conclusion is supported by *in vitro* mobilization experiments. *In vivo* survival of EK2 strains and their potential transfer of either conjugative or non-conjugative plasmid must be considered to be below the current limits of experimental detection.

3. *E. coli* K-12 strains which survive passage through the human or animal gut rarely show evidence of having acquired plasmids from the indigenous flora. In calf-feeding experiments, only 1 out of well over 10,000 K-12 strains which were screened had acquired colicinogeny.

4. Over a period of 26 months in Bristol, 12 months in London and 12 months in Seattle involving 64 subjects, there was no evidence that laboratory workers, or members of their families, acquired either bacterial strains or plasmids that were employed in the laboratory. This was true even in a few individuals working in the laboratory who received short courses of antibiotic therapy. Monitoring of the oropharynx failed to show any evidence of colonization by laboratory strains. Individuals with excessive facial hair likewise showed no evidence of carriage. In a few instances, individuals who had done routine experimental procedures (vortex mixing, or plating, etc.) showed transient numbers of *E. coli* K-12 on their laboratory garments, facial skin or hands. These organisms were few in number (4/cu$^{mm^2}$) and disappeared within three hours. Simple washing with soap and water resulted in instantaneous loss of these organisms. No evidence whatsoever was found for even transient survival of *E. coli* EK2 derivatives.

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[2] The following Report was presented by M.H. Richmond to the Meeting of the Risk Assessment Subcommittee of COGENE which met at The Royal Society, London, on Friday, March 30th, 1979. The Report was compiled by Dr. Stanley Falkow on the basis of information provided by himself, by E.S. Anderson and by M.H. Richmond. The information contained in the Report represents the situation as it was at the end of October 1978, save that the data from Bristol have been updated to include information up to the end of 1978.

5. Monitoring of the laboratory work bench, water baths, and centrifuges, etc., occasionally revealed detectable numbers of EK1 *E. coli* K-12. Such organisms could be found immediately after performing laboratory procedures such as flaming bacteriological loops, replica plating or transferring cultures (even with manual pipetting devices). Such contamination was instantaneously sterilized by simple disinfectants, including 70% ethanol. Deliberate spills of EK1 *E. coli* K-12 had become sterile within six hours after drying. Reliable numbers for the survival of EK2 hosts in the laboratory are not available to us but survival, if any, was much reduced relative to ordinary *E. coli*.

6. Individuals working with *E. coli* K-12 infected mice and calves were not found to more likely become colonized with *E. coli* K-12 than workers who did only *in vitro* experiments.

DISCUSSION

S.D. EHRLICH: I wonder whether there is a figure available on the cost of the risk assessment experiments up until now?

A.M. SKALKA: The cost of the risk assessment experiments?

S.D. EHRLICH: I'd like to have a global estimate on the amount spent on risk assessment experiments since the recombinant DNA question was raised.

A.M. SKALKA: I don't have that figure. What information do you hope that would provide?

W.J. GARTLAND: I wanted to mention that when the NIH Guidelines were issued on December 22nd, there was a directive to NIH to conduct a risk assessment programme. The directive says that further actions under the Guidelines must be based on data gathered from a risk assessment programme. NIH has had an internal working group which has proposed a risk assessment plan which is about to be published in the *Federal Register*. The proposal lists some types of things that could be done. One of the central features of the proposal is the recruitment of an eminent scientist who would come to the NIH and who, in addition to directing the programme, would do a lot of data-gathering to try to pull together a lot of information which is already in the literature and perhaps prod people to do some simple risk assessment studies. The proposed plan will be published for comment very shortly.

D. HABER: This is something I'd like to ask someone from the NIH about because it's just a rumour I've heard, so it may or may not have any foundation. I have heard that there was a meeting of an NIH subcommittee on vectors on March 19th at which some evidence was provided - and this evidence is supposed to have been put forward by NIH Private contractors - that EK2 microorganisms survive in sewage. I wonder if someone from the NIH could elaborate on that - confirm, deny, or explain it.

ANNA MARIE SKALKA: Yes, the contractors were Drs. Sapik, Walter and Sorber. The report is available for you to look at and it is included as an appendix in my report.

D. HABER: I would like to direct myself again to the NIH and to the risk assessment activities of COGENE, in particular of EK2 strains K-12. I wondered what note is being taken, or advice being given, or if, in fact, there has been any consideration of evidence that the properties of the EK2 organisms seem to change when grown in large quantities - or could possibly change? Some evidence has been published to that effect and I wonder whether that's been considered.

INDUSTRIAL PRACTICES SUBCOMMITTEE of the FEDERAL INTERAGENCY ADVISORY COMMITTEE on RECOMBINANT DNA RESEARCH

COMMENTS: CHRISTINE OLIVER, M.D., M.S., for the OIL, CHEMICAL and ATOMIC WORKERS INTERNATIONAL UNION

JUNE 18, 1980

I want to thank the Subcommittee for its invitation to attend the meeting today to discuss the concerns of workers as industry prepares for the large scale application of recombinant DNA technology in commercial production. Anthony Mazzocchi, Director of Health and Safety for the Oil, Chemical and Atomic Workers International Union (OCAW), was not able to attend; so I am speaking in his place. I only hope that the allocation of 15 minutes to each speaker is not an indication of the seriousness with the Subcommittee intends to take the comments. It is impossible to enumerate, consider and discuss the issues involved in that period of time. I will attempt to givd an overview of the problem based on the OCAW's long and bitter experience with the industrial sector and on my own more limited experience as a physician working for the Union.

The OCAW represents 180,000 of the more than 97 million workers in this country. Our members are employed in the oil, chemical and atomic industries; many of those employed in the chemical industry work in pharmaceuticals and petrochemicals. The Union has a strong health and safety department and a long history of activism in the public health and safety arena. The majority of workers in this country do not have the benefit of union organization and support; many of them work in small, antiquated shops. Many of these shops are chemical factories. Some of them will eventually use recombinant DNA technology as commercial application expands. There is no health and safety spokesperson for these workers.

The concerns of the OCAW for its own and other potentially exposed workers as recombinant DNA technology moves from the laboratory into the industrial sector are several. First, workers at the point of production will be the first to experience the risks of this biotechnology. The health hazards now faced by our members and other industrial workers are many and varied. What does not vary is the fact that there are certain fundamental characteristics of workplace policy and practice that produce exposure to toxins and carcinogens at the job site. These include the following:

1. The workers' Right-to-Know is ignored. Information essential to their self protection is not given to them: the generic names of the substances with which they work; the results of environmental monitoring of levels of expoxure; the results of toxicity data to which the company has or should have access; group morbidity and mortality data.

2. Environmental monitoring for exposure to toxins and carcinogens is at best sporadic. Biological monitoring for the physiologic effects of these exposures is more systematic but often of questionnable quality; and the results are often interpreted by a physician more interested in preserving the health of the company than the health of the worker.

3. Engineering controls are inadequate to control exposures. If they are sufficient under routine working conditions, they are rarely able to protect workers in the event of an accident or spill.

4. Labelling of vessels and pipes containing toxic materials is inadequate to nonexistent.
5. Morbidity and mortality data that is collected is rarely analysed in a systematic or epidemiologic fashion. Consequently, clusters of disease resulting from specific exposures go undetected until late in their course. And prevention is obviated.
6. Worker education programs are confined to larger companies, for the most part. These programs are usually sporadic. They deal primarily with safety rather than health issues and with acute rather than chronic effects.

The industrial operation that produces biological products and uses biotechnology is no different from the industrial operation that produces chemical, metal, fibrous and other types of products. Hazardous exposures of workers to the byproducts of production and subsequent illness and disease will result from the same causes: inadequate engineering devices, failure of control systems, lack of information on the part of the worker, failure to label and failure to monitor--the environment or the worker.

Second, screening for the "hypersusceptible" worker is now a common practice in many plants. The expanded use of recombinant DNA technology introduces the possibility of genetic manipulation of this "hypersusceptible" worker to modify him or her to make him/her compatible with the work environment. Thus the workplace itself does not have to be cleaned up.

Third, the use of this technology for commercial production, particularly in the chemical and mining industries, may well result in significant job loss for our own members and other workers as well. The economic and health effects will be quite serious, not only for these workers but for the country as a whole.

The NIH Guidelines for Recombinant DNA Research do not adequately address these issues. Compliance by industry is purely voluntary. It is inappropriate to have the same agency both promoting and regulating the use of any technology. And the Recombinant DNA Advisory Committee (RAC) has proposed (Federal Register, April 30, 1980, pp. 28904ff) the amending of the Guidelines to require membership by industry representatives. This state of affairs has been made worse by the recent Supreme Court decision to allow the patenting of microorganisms. Industry is thus being encouraged not only by their own self-interests but by government agencies and the federal judiciary to vigorously pursue the use of recombinant DNA technology for large scale commercial production. Under these circumstances the voice of the worker will never be heard; and the health and will-being of the worker will not be protected.

Before the use of this technology for commercial production expands further, the development of control technology and workplace practices that will maximally protect the worker is essential. In addition, regulations that govern industrial use and application of recombinant DNA technology must be in place before rather than after the fact of commercial production. And these regulations must have the force of law behind them, with appropriate penalties for violation, if they are to be effective. The appropriate research and development agency is NIOSH; the appropriate enforcement agency, OSHA. To whom should fall the task of certification of industrial use of specific recombiannt DNA systems is less clear. In all three areas, however, meaningful input from labor representatives and from workers at the point of production is essential. A single labor spokesman on the RAC and 30 minutes before this subcommittee can not be considered meaningful input.

Regulations that are developed, whether by OSHA, by Congress or by an alternative group or agency, must address the following issues:

1. Right-to-Know. Workers have the right to know what they are working with, in what quantities and with what known or predictable consequences. Without this information, the workers can not provide themselves with adequate protection guaranteed under the law.

2. Environmental monitoring. Techniques of environmental monitoring should be developed in conjunction with the National Institute for Occupational Safety and Health and the Occupational Safety and Health Administration; and these techniques should be applied in a systematic and routine fashion within both the industrial production and the industrial research and devlopment facilities.

3. Biological monitoring and medical surveillance. Biological monitoring and medical surveillance must likewise be carried out in a systematic and ongoing manner.
   a. All companies using recombinant DNA technology should be required to keep OSHA health and safety log forms relevant to the hazards of thes technology.
   b. National standards for medical surveillance programs specific for each class of organism and group of experiments should be developed by NIOSH and enforced by OSHA.
   c. All workers potentially exposed to recombinant DNA material should have the opportunity to participate in such surveillance programs with guaranteed economic and seniority protection. Members of their families should be similarly evaluated when appropriate.
   d. A central registry for recombinant DNA -process associated illness and disease, proven or suspected, should be established and maintained with NIOSH.
   e. Epidemiologic analysis of morbidity and mortality data should be carried out as it accumulates; so that trends can be detected quickly.

4. Emergency spills. A set of national standards should be developed for handling emergency spills, not only to protect the worker but also to protect the general public.

5. Worker education. Relevant and comprehensive worker education programs must be developed and required for all workers potentially exposed to recombinant DNA materials.

In summary, chemical hazards have not been eliminated from industrial production; nor have they been adequately controlled. Biological hazards are different only in that they are more difficult to monitor. Accidents are inevitable. As commercial use increases, production will increase. Job speed-up will result. Accidents will increase. And hazardous exposures will increase. These hazardous exposures will affect not only the workers themselves, but also their vamilies and the public at large. Well known examples are asbestor and lead. And like other occupational and environmental disease, these will in many cases be subtle and difficult to detect without careful scrutiny. For example, urinary tract infections and diarrhea are common illnesses. Unless causal organisms are carefully classified and compared to those in use in a laboratory or factory, the occupational link will go undetected.

The Oil, Chemical and Atomic Workers International Union believes that adequate protection of the worker from harmful exposures to recombinant material is impossible.

To broadly apply recombinant DNA techniques in the commercial sphere opens the door to a kind of genetic manipulation that violates ethical, religious and political tenets formerly held sacred. If such activity is going to continue, however, Labor must have meaningful and ongoing input into its regulation if any degree of worker and public protection is to be achieved. A first step would be for members of this subcommittee to meet with workers at the actual point of production to discuss with them (in a setting that allows them to speak freely) the workplace as they perceive it--both in terms of exposures, of education, of access to information and of health care received at the plant. The experienced of the workers in general industry is extensive and applicable to this "novel" mode of production.

STATEMENT
OF
ELI LILLY AND COMPANY
TO THE
INDUSTRIAL PRACTICES SUBCOMMITTEE
OF THE
FEDERAL INTERAGENCY ADVISORY COMMITTEE
ON
RECOMBINANT DNA RESEARCH

Presented by:

Irving S. Johnson, Ph.D.
Vice President of Research
Lilly Research Laboratories

Wednesday, June 18, 1980

Mr. Chairman and Members of the Committee, I am Irving S. Johnson, Vice President of Research, Lilly Research Laboratories. I have administrative responsibility at Lilly for biological research directed toward pharmaceutical products for human medicine, including work in recombinant DNA research. I am accompanied by Bernard D. Davis, M.D., Adele Lehman, Professor of Bacterial Physiology and Director of the Bacterial Physiology Unit, Harvard Medical School.

Lilly has been engaged in the research, development, production, and distribution of pharmaceutical products for many years and of agricultural products for a number of years. The company has a strong commitment to research and has at present in excess of twenty-six hundred scientists and technicians involved in its research and development programs. Their activities have made possible the development of a wide variety of products which have benefited both human health and agriculture.

My remarks and comments will be addressed to possible or potential occupational hazards which may or may not be involved in industrial scale recombinant technology.

Lilly supports the program of voluntary compliance with the NIH Guidelines for Recombinant DNA Research for non-federally funded research. Historically, Lilly observed the moratorium which applied to certain types of recombinant DNA research, and after the establishment of the NIH Guidelines, has voluntarily complied with them. As you know, the NIH Guidelines require approval of all protocols by an Institutional

Biosafety Committee (IBC) which must have outside membership of at least two or twenty percent of the members. Lilly has an IBC of nine members of whom three are outside members. I act as chairman. They include the Mayor of the 11th largest city in the United States, who is a former member of the Congress, and a minister of the Presbyterian Church. One could say he represents philosophy and ethics as well as the local community while being experienced and familiar with the importance of compliance and cooperation with Federal agencies. The other members include the Vice President of Indiana University, who is both a physician and educator; and a pathologist, expert in diagnosis. He is also Chairman of the Infectious Disease Committee of the Indiana University Medical School. We have kept the community, our stockholders, and our employees informed of our intent and involvement in recombinant DNA research as described in congressional testimony (1,2).

In considering potential occupational hazards in this area I will comment on five principle items. These are:

(1) The origin of the 10-liter (10L) limitation.

(2) The safety of E. coli K12 as determined by recent risk assessment programs.

(3) The reliability and operating procedures of commercial scale fermentation equipment including training of operators.

(4) Medical surveillance program for equipment operators.

(5) The current authority of OSHA and the FDA.

Origin of 10L Limit

The origin of the 10L limit appears to have evolved at Asilomar during a period when few people conceived of performing large-scale fermentation with recombinant organisms. Dr. Stanley Cohen (3) described the main factor which was considered, namely that 10L was probably as large a volume as could be conveniently handled in an experimental laboratory by conventional laboratory centrifuges. More recently, Dr. David Baltimore (4) suggested that 10L was considered as a standard volume representing a "large amount" in a laboratory. There was a very conscious understanding that it excluded industrial-level activity. It was never suggested that increased volume of a culture of a safe organism resulted in any increased risk.

Safety of E. coli K12 as Determined by Risk Assessment

Recombinant DNA technology is the culmination of a number of basic discoveries in molecular biology during the preceding quarter century. During those years scientists learned that DNA was the storehouse of genetic information in all cells, and following the elucidation of the chemical structure of DNA, understood the manner in which this information was expressed and controlled in microorganisms. In the 1960's and early 1970's, methods of cutting up DNA molecules and rejoining them were developed, along with ways of introducing these molecules into functional cells. Although these discoveries led some scientists to question whether indiscriminate insertion of new genetic information into cells might lead to public health and environmental risks, these same discoveries have been directly responsible for an exponential increase of new knowledge in molecular biology.

Since that time various "risk assessment" experiments, as well as deliberate attempts to test the "hypothetical risks," have been undertaken. The most extensive of these were sponsored by the National Institute of Allergies and Infectious Disease (NIAID), utilizing scientists expert in molecular biology and infectious disease both in this country and overseas. Three areas of conjectural concern have been addressed in the NIAID program relative to E. coli K12 hosts. The Falmouth Workshop (5) dealt with the potential innate pathogenicity of the organism. The polyoma experiments at the NIH (6,7,8,9) as well as those in the United Kingdom (10) and the deliberations of the Ascot Workshop (11) dealt with a "worst possible case" scenario of the organism containing pathogenic DNA (i.e., oncogenic or cancer causing). Recently, the Pasadena Workshop considered two issues. Both concerned colonization of the gut with an organism producing a biologically active hormone. One issue was the magnitude of the inherent pharmacological effect of the hormone and the second issue dealt with sequelae which might result due to immunogenicity of the hormone. The overwhelming conclusion from these and other risk assessment data is the unquestionable safety of E. coli K12 hosts utilizing non-conjugative plasmids with carefully and fully characterized DNA inserts. I feel these conclusions are applicable not only to research and pilot plant stage fermentation but also to commercial scale production. These results justify the concept of biological containment as well as physical containment embodied in the original Guidelines of June 23, 1976. Current recognition of the decreasing risk of recombinant DNA research is further exemplified by the action of the Recombinant Advisory Committee (RAC) on September 6-7, 1979, recommending the exclusion of all E. coli K12 research (approximately 80 percent of all recombinant DNA research in the U.S.) from the Guidelines, except for those experiments over 10L. The Director elected to keep this research at P1-EK1 but did give full authority for its approval to the local IBC. At the RAC meeting of June 5-6, 1980, however, the RAC recommended approval of an amendment to the Guidelines which deleted the following phrase from Section I-D-6, "We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale" by a vote of 14 yeas, 1 nay, and 3 abstentions. The nature of equipment used for large-volume fermentations is such that increases in volume do not pose any increase in the problem of containment; if anything, the probability of hypothetical risk is diminished in proportion to the number of runs necessary to provide a target total volume per unit time. We commend the RAC on its acceptance of this deletion.

Commercial Scale Fermentation Procedures and Operator Training

I would like to comment briefly on these large-scale procedures and the reliability of the systems. Although in recent years many technical changes have occurred in the age-old process of fermentation, the principles for fermentation of recombinant DNA organisms are essentially the same as those used for antibiotics since the 1950's. The basic equipment consists of closed stainless steel vessels designed, built, and tested to comply with pressure vessel codes. Such a vessel is normally fitted with an agitator to mix the contents and aid in aeration. Compressed air which has been filtered to remove contaminating organisms is pumped into the fermenter to provide necessary oxygen for growth. Metabolic heat is removed by a cooling jacket. Modern instrumentation monitors and controls variables such as fermenter temperature, air flow, pressure, pH, dissolved oxygen, feed rates of various nutrients, and composition of exhaust gases. Operating pressures are modest and are nominally about 5 psig.

Antibiotic fermenters are designed primarily to protect the fermentation from contaminating organisms. To adapt them to fermentations with recombinant organisms, we have modified them to contain the fermentation organism more completely. This was readily accomplished by filtration or incineration of the exhaust gases, containment of the agitator seal, sampling and inoculating with a closed system, and followed. by thermal or chemical sterilization of the fermenter contents before processing. Detailed operating procedures are used and the equipment and procedures are thoroughly validated before the system is used for fermentations with recombinant organisms. A schematic representation of both types of fermenters is attached.

Monitoring of the operating system is carried out continually and consists of microbiological sampling of the exhaust systems after filtration, sampling of the operator environment, and frequent integrity testing of the filtration systems. In over 2,000 hours of operation with fermentors containing recombinant organisms, we have never recovered the recombinant organism from the enviornment even though it is regularly monitored. After thermal or chemical kill, a sample of each fermentation batch is plated on a culture medium to monitor effectiveness of the procedures.

The protocols required to operate fermentation vessels with microorganisms containing recombinant DNA are somewhat more complex because of the containment features for inoculation, sampling, etc., but are readily learned by skilled pilot plant and production operators. These operating protocols have also incorporated a number of redundancy features to minimize inadvertent breach of containment.

The training of fermentation operators has included a general introduction to all aspects of recombinant DNA and containment issues, and highly specific training for everyday operation. The general training has included viewing the 1976 Nova film on recombinant DNA which discusses the benefits and potential risks associated with recombinant DNA research as they were perceived through 1976. Subsequent to viewing the film, various aspects of recombinant DNA have been discussed by the biological safety officer, the engineering coordinator responsible for fermenter design, and immediate supervisors. Operators have been encouraged to participate in discussions on potential risk, containment and the NIH Guidelines. Detailed specific training on the pertinent aspects of the NIH Guidelines and on operating protocols designed to meet the specifications of the Guidelines and to avoid loss of containment have been carried out by principal investigators and the operators' supervisors. The operators have been encouraged to discuss any problems or technical questions with the principal investigators, supervisors, biological safety officer, or physicians in industrial medicine.

Medical Surveillance

Because of our long-standing interest and responsibility for the health and well-being of our employees, we have for a number of years carried out programs of medical surveillance of workers in both laboratory and production areas. The surveillance program has been altered slightly for workers in recombinant laboratories. Our program includes the following procedures:

1. Medical questionnaire and standard periodic physical examination including complete blood count, urinalysis, multicomponent blood chemical analysis (SMA 12), and posterior-anterior chest x-rays on an annual basis.
2. Baseline frozen serum samples are obtained and maintained on all employees working with recombinant DNA for future comparative analysis, if deemed necessary.
3. All breaches in containment are recorded and reported immediately to the Biological Safety Officer and a physician in industrial medicine for appropriate action which may include monitoring for the development of any illness.
4. All illnesses must be reported to supervisors.
5. Any employees who are off work for illness for more than five days must report to a physician in industrial medicine before being allowed to return to work.
6. Any employee taking antibiotics is restricted from an area of recombinant DNA research until five days after the discontinuance of the antibiotics.
7. A computer listing of all workers involved in recombinant DNA activities is maintained and updated as required.

The preventive medicine and health surveillance aspects of these programs are available to all Lilly employees and are mandatory for laboratory and production workers.

Factors involved in several of these areas provide a series of redundancies for safety. The 10L limitation as (opposed to 9.2L, 9.5L or 500L) was an arbitrary figure selected without consideration for industrial application. The most widely used organism at the industrial level is _E_. _coli_ K12. Laboratory strains of _E_. _coli_ were originally selected for recombinant work because it was the single microorganism which was best understood genetically, and any concerns about risk associated with it were largely based on the fact that _E_. _coli_ was a normal component of the flora of the intestinal tract in man and animals. It has been demonstrated repeatedly that there is no significant colonization capacity for K12 and several of its derivatives. Having reestablished that fact for any modified _E_. _coli_-K12-EK1 host-vector system, other risk studies taken out of context have no real significance.

Moreover, industrial scale fermentation of a wild type _E_. _coli_ has been going on for more than thirteen years with no evidence, to our knowledge, of any occupational health problems and Lilly has had direct experience with this fermentation with no problems. I am enclosing a preprint of a historical review of this industrial experience which was presented at the recent FDA Symposium on the State of the Art: Insulin and Growth Hormone.

By all available risk assessment data, E. coli K12 is safe and non-pathogenic. Modifications, if any, have been in the direction of making the host more dependent on the support of its laboratory environment. The plasmids are always fully characterized--i.e., their genomic sequence is known; they were selected initially because they were of the non-conjugative, poorly mobilizable variety. The safe and non-pathogenic organism is fermented in a rigid, stainless steel closed vessel. Prior to harvesting and chemical extraction, the safe, non-pathogenic organism is chemically or thermally killed.

In the future, one may expect that other host organisms will be used industrially--for example, streptomyces or saccharomyces. Again, the criterion of inability of the laboratory-modified strains to survive in their natural habitat should be met. We must examine new vectors selected for large-scale fermentation studies, to determine whether or not they modify their host organism's behavior. These organisms offer the obvious advantage of not having any normal interaction with humans or animals.

The NIH has now published recommendations on physical containment for large-scale recombinant fermentation systems to be used as a guide by commercial firms. Lilly supports these and made recommendations during their development. At this point in time, sufficient knowledge exists to assure reasonable decisions on the design, construction and operation of industrial facilities for production of materials by recombinant methods. The exercise of that knowledge can occur at the local level of review in the same way as for any complex technological advance in American industry. The NIH Guidelines for Recombinant DNA Research have provided a prudent approach to applications of the technology.

Authority of OSHA and FDA

It is my feeling that the RAC, in its certification of an experiment as being free of harmful gene sequence and recommending a level of physical containment, has rendered an adequate judgment on safety. As we move to pilot plant and commercial scale fermentation of these organisms, I believe existing agencies represented on this subcommittee already have adequate authority to monitor any additional problems, if they arise. Moreover, it is my impression that they are already functioning. Lilly's effort in recombinant technology, for example, has already been inspected by a subcommittee of the RAC, a four-member NIOSH group, including Mr. Pauker, and a two-member group from the FDA. A report of the RAC inspection is attached.

OSHA has adequate authority under the Occupational Safety and Health Act of 1970, to inspect industrial facilities and conduct investigations relating to worker safety. Further, OSHA has promulgated standards for safe industrial practices.

Additionally, the FDA will approve for safety and efficacy any pharmaceutical product which results from recombinant DNA research. Consequently, both the pharmaceutical manufacturer and its products are extensively regulated.

In view of the safety of the organism in use, the risk assessment programs which have been conducted and the degree of regulation already in place, it would appear appropriate for the RAC to continue to exercise oversight over recombinant DNA research in a manner which will facilitate rational technological progress without arbitrary and unnecessary restraints.

Finally, I fully support one set of rules for recombinant research. I hope the subcommittee will support one mechanism of oversight in this area for laboratories in industry, academic institutions and government facilities. Recombinant research is performed in all three types of laboratories and each group should be treated equally and fairly.

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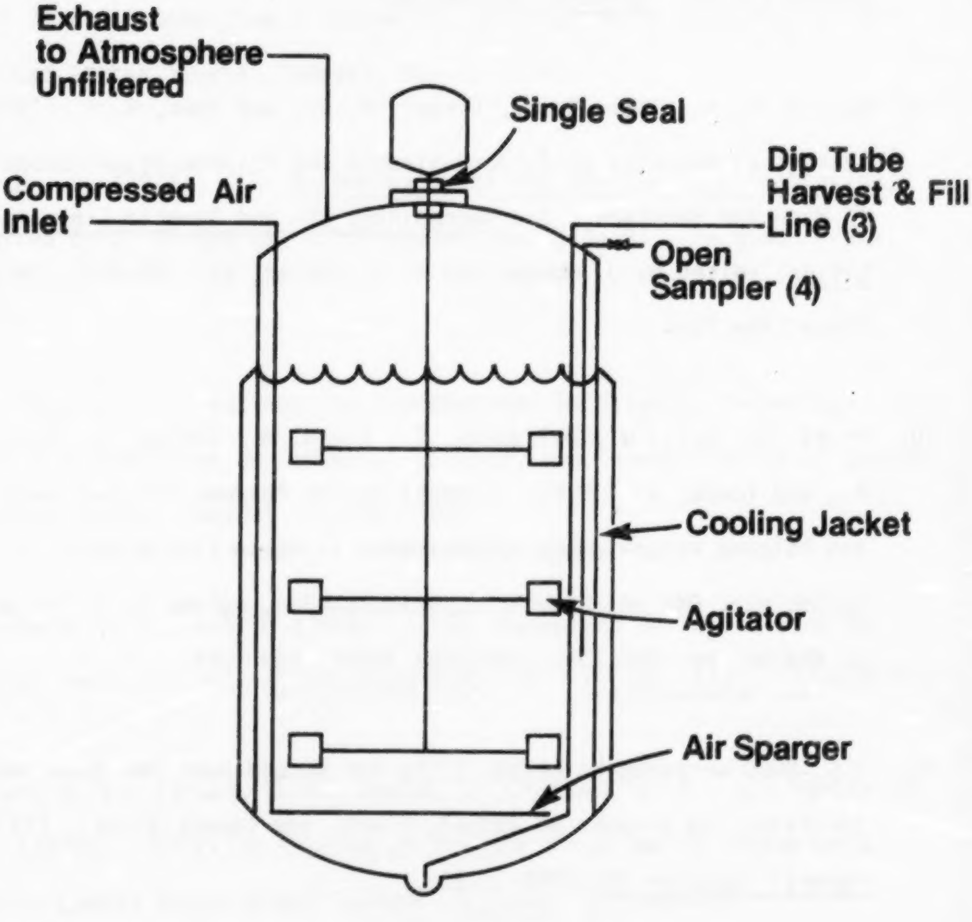
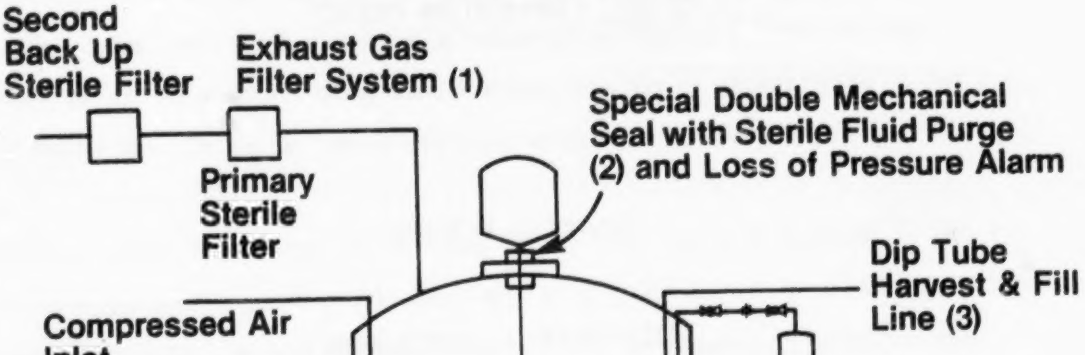
Features of Standard Fermentor


Exhaust to Atmosphere Unfiltered
Single Seal
Dip Tube Harvest & Fill Line (3)
Compressed Air Inlet
Open Sampler (4)
Cooling Jacket
Agitator
Air Sparger

Features of Contained Fermentor
Eli Lilly 2,000 Liter Fermentor


Second Back Up Sterile Filter
Exhaust Gas Filter System (1)
Special Double Mechanical Seal with Sterile Fluid Purge (2) and Loss of Pressure Alarm
Primary Sterile Filter
Dip Tube Harvest & Fill Line (3)
Compressed Air Inlet
Stainless Steel Sterilizable Sampler (4)
Cooling Jacket
Agitator
Air Sparger

Other Features

(6) Collection of Contaminated Condensate and Sterilization before Release

(7) System Validation before Operation

(8) Monitoring Program

(9) Operation with Detailed Protocol

L-ASPARAGINASE: A CASE STUDY OF AN E. COLI FERMENTATION PRODUCT

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L-asparaginase (L-asparagine amidohydrolase) is the first enzyme to be applied successfully to the treatment of cancer. This protein from Escherichia coli, with a molecular weight of 130,000 daltons now has a 12-year record of usefulness for the induction of remission in the acute lymphatic leukemias, especially those of childhood. The systemic administration of asparaginase kills tumor cells that are dependent on L-asparagine, and, that also have an impaired ability to synthesize that amino acid. As with all other cancer drugs, the use of asparaginase is not without risk. It has an innate toxicity which must be considered.

Of more immediate interest to us today is the fact that asparaginase is the first example of a commercially available E. coli fermentation product to be used clinically. We are now concerned with the development of proteins such as human insulin and growth hormone, derived from E. coli by recombinant DNA technology. What problems will we have with contamination by extraneous bacterial proteins and lipopolysaccharides such as endotoxin And, what are the clinical implications Bacterial contaminants, even at a relatively low concentration, conceivably could give rise to objectionable side reactions of an immunologic nature. How serious were such problems, and how were they resolved during the development of asparaginase What lessons can we learn from the experience with asparaginase that could be applied to our present situation These are questions that are relevant to this conference. The concept of administering human proteins derived from bacteria has the connotation of unknown risks. Perhaps in considering the example of asparaginase we may be able to assess at least some of those risks more objectively, and place them in proper perspective. I would like to review asparaginase as a case study of the use of a protein from E. coli in humans and emphasize primarily the side effects and clinical toxicity of enzyme preparations in which bacterial contaminants were at least a contributory factor.

Certainly, the first preparations of asparaginase to be used clinically in 1967 were crude, even by the standards of protein purification at that time. However, the clinical effectiveness of these crude preparations in reversing a life-threatening situation justified their use and the toleration of a moderate degree of side effects. A hypothetical curve of the specific activity of clinical trial lots of asparaginase during the first three-year period of evaluation of this enzyme would look similar to that depicted in Figure 1. Early lots of the enzyme had specific activities in the range of 25 to 35 International Units per milligram, roughly 10 percent of the specific activity of the highly purified material made available by 1969 and 1970. Although the specific activities of these early lots of enzyme were low, this curve does not imply that these preparations were contaminated by 80 to 90 percent of extraneous bacterial components. The enzyme was readily inactivated or denatured during isolation, and a major part of these first preparations may have consisted of inactive forms of the enzyme.

The total time to progress from crude to highly purified preparations of asparaginase was about two and one half years. Contrast this with the slow evolutionary process of insulin purification spanning half a century. Of course, the state of the art in natural product purification and characterization, the analytical capability to recognize contaminants in both a qualitative and quantitative sense, and the awareness of clinical implications of contaminants, make the difference in time. Today, we recognize that products of recombinant DNA technology will be at a very high degree of purity the first time they are used clinically.

In considering the toxicity of asparaginase lots, it is difficult to distinguish between certain toxic effects arising from the pure enzyme, per se, and those caused by bacterial contaminants. In Table 1, I have attempted to classify the toxic effects into the two groups. From the clinical point of view, pancreatitis, hepatotoxicity, and occasionally severe hypersensitivity reactions, such as anaphylasis, were the more important toxic manifestations. Pancreatitis was relatively rare and was dose related, but when it did occur it was usually of a fulminating, life threatening nature. Chills, fever, nausea and vomiting shortly after drug administration were definitely related to bacterial contaminants. Hypersensitivity reactions occurred with both crude and purified preparations, and investigators were unable to differentiate between these with respect to incidence. It would be ideal for our purpose to have a comparative toxicity study of crude versus highly purified preparations of the enzyme in order to have a better understanding of toxicity due to contaminants. The nearest approach to this is the report of Dr. Herbert Oettgen and associates at the Sloan-Kettering Institute, concerning the toxicity of E. coli asparaginase in man (3). Pertinent information from the protocol of this study is shown in Table 2.

This study summarizes the Sloan-Kettering experience over the early and mid-phases of clinical evaluation of the enzyme during the years 1967 to 1969. The enzyme was obtained from four suppliers during that period and there was a 10-fold range of specific activities of the batches. The enzyme was administered by the usual daily intravenous route, and three dose levels were used: 200, 1000 and 5000 I.U./kg body weight. Depending on the specific activity of the preparations, this dose range was equivalent to about 5 to 18 milligrams of protein per kilogram. The duration of treatment was four weeks and a total of 274 patients was studied.

While many of the early lots were pyrogenic, it was considered that the probable contamination by endotoxin was not sufficient to cause serious clinical problems. In animal toxicity studies no clear-cut evidence of endotoxemia was observed even with crude preparations of asparaginase. Batches were accepted for use if the pyrogen assay in rabbits showed a mean temperature rise of not more than 1.5° centigrade in three rabbits. Temperatures of this magnitude are induced by endotoxin doses two to three orders of magnitude below lethal doses. The investigators attempted to correlate low, that is, less than 70 I.U./mg versus high, greater than 150 I.U./mg specific activity lots.

Clinical observations of side effects in which bacterial contaminants may have played a major role are listed in Table 3. There was good correlation, as a rule, between specific activity and pyrogenicity of the lots; the lower purity lots were generally more pyrogenic. There was also a good correlation between the results of the pyrogen test in rabbits and the occurrence or absence of fever in the patients. While many of the patients were febrile before therapy with asparaginase was started, there was a 30 percent incidence of fever that was attributed to the enzyme, and the low purity lots produced a greater incidence of fever.

Anorexia, nausea, and vomiting were experienced by about half the patients; again, there was a rough inverse correlation with specific activity, and also a direct correlation with dosage. A 30 percent incidence of immune reactions of various kinds was observed during the first course of treatment. As might be expected, a somewhat higher 41 percent incidence was found when second courses of treatment were instituted at a later time. Antibody formation to the enzyme preparations, as determined by immunodiffusion and complement fixation, occurred in about 70 percent of the cases. Cross reactivity among the products of the four suppliers was evident. The Ouchterlony tests indicated that antibodies were directed against several antigenic determinants. Urticaria was the most common clinical symptom of hypersensitivity. Conclusions drawn from this study that are especially relevant to the bacterial contaminants are summarized below.

1. Treatment with *E. coli* asparaginase produces a variety of similar toxic side effects in animals and in human patients. While certain of the side effects can be attributed to bacterial contaminants, the enzymatic activity and the immunogenicity of this foreign protein are the major factors in the toxic manifestations observed.

2. While fever was a problem with the earlier batches that contained large amounts of impurities, fever is rarely of concern with purified preparations of the enzyme.

3. Nausea, vomiting, and anorexia also occurred more frequently when pyrogenic preparations were used. Endotoxin was probably a contributory factor in their pathogenesis.

4. With regard to immunogenicity, these investigators state: "Perhaps the most surprising thing in this respect was that the administration of an *E. coli* protein over a long period of time was not more troublesome."

Even with the crude preparations used in this study and the second course of treatments given to some patients, there were no fatal anaphylactic reactions.

Concerning endotoxin contamination of *E. coli* asparaginase, it is of interest that Loos and associates at the National Cancer Institute found that thirteen out of fifteen asparaginase lots that they examined gave an appreciable inhibition of whole human serum complement, an activity associated with endotoxin (2). The anti-complementary activity could be completely separated from the enzyme activity by gel filtration on Sephadex G-200 as shown in Figure 2. Fractions containing the asparaginase activity had no effect on whole human serum complement, or on the first component of complement. The anti-complementary fraction was shown to be antigenically related to E. coli lipopolysaccharide or endotoxin, and had essentially no anti-tumor activity.

I would like to summarize briefly the experience of Eli Lilly and Company with L-asparaginase from E. coli B. We began producing it experimentally on a very small scale in 1968, and by July of that year had a preparation which we thought would be suitable for clinical evaluation. Some of the safety testing of this material which is pertinent to our discussion is shown in Table 4. This lot was produced before we learned how to crystallize the enzyme and the specific activity was only 132 I.U./mg. I think the important data from this table are that the material was essentially non-pyrogenic and had no detectable endotoxin within the limits of the assay. This material was not used clinically as we were already starting to crystallize the enzyme at that time and we opted to go to the clinic only with the purified crystallized enzyme (1) as shown in Figure 3. The process we finally developed for clinical trial lots of asparaginase is summarized in Table 5. It was a straightforward purification procedure consisting chiefly of ammonium sulfate and ethanol fractionations followed by two crystallizations from ethanol-water mixtures. No Sephadex or ion exchange chromatography was used.

Polyacrylamide gel electrophoretic patterns of asparaginase are shown in Figure 4. Gel number one shows the ethanol precipitated enzyme from step 4 of the previous figure. Gel 2 is the pattern of the enzyme immediately prior to crystallization. Gels 3 and 4 are two preparations of the crystallized enzyme. These gels were loaded with 100 micrograms of protein each. The crystallized asparaginase was non-pyrogenic in rabbits at a dose of 5000 I.U./kg. This is equivalent to about 17 mg/kg.

The marked increase in specific activity across crystallization which amounted to about a three-fold purification suggested the possibility that the crystallized enzyme might offer some therapeutic advantage over amorphous preparations of asparaginase that were then being evaluated clinically. Purification of the enzyme by crystallization rather than by chromatography also appeared to be a favorable factor for the scale-up to commercial production. Accordingly, acute toxicity studies were conducted on the material appearing in gels 2 and 3, that is, the material before and after crystallization. The specific activities of these particular preparations were 135 and 275 I.U./mg respectively, approximately a two-fold difference in purity. This comparative acute toxicity study was performed at the suggestion of the Division of Biological Standards which was the FDA bureau responsible for regulatory aspects of asparaginase at that time. Four species were used in the acute toxicity study: mouse, rabbit, dog and monkey. There were no significant differences in acute toxicity of the two preparations, except that the cruder material induced a weak *in vitro* hemolysis of canine erythrocytes. The LD_0 in both the dog and the monkey was greater than 16,000 I.U./kg body weight; this is equivalent to more than 120 mg protein/kg in the case of the cruder preparation.

A 14-day subacute toxicity study of crystallized asparaginase was conducted in the dog and monkey at doses of 200, 1000 and 5000 I.U./kg body weight, and in the rabbit at 50, 100 and 200 I.U./kg. The rabbit had been found by other groups to be very sensitive to asparaginase toxicity, and our study confirmed this. A one-month subacute toxicity study in the dog and monkey was then done at 1000 I.U./kg. Toxicity encountered in both of these studies with crystallized asparaginase was relatively mild, and no serious toxicity was observed which would contraindicate a cautious clinical trial of the enzyme. Subsequently, clinical evaluation of our crystallized asparaginase was done by groups at St. Jude Children's Hospital, Memphis, and at the Sloan-Kettering Memorial Hospital, New York. In brief, no significant differences in clinical toxicity or efficacy were observed for the crystallized enzyme over other purified asparaginase preparations.

In summary, crude preparations of _E_. _coli_ L-asparaginase produced side effects such as chills, fever, nausea and vomiting which appeared to be associated with contamination by pyrogens and bacterial endotoxin. These particular side effects were minimal when highly purified preparations of the enzyme were administered. However, the principal toxic effects were observed with the purified enzyme as well as with the cruder preparations. The immunogenic nature of this large, foreign protein of 130,000 daltons and the enzymatic activity, _per se_, both asparaginase and glutaminase activity, comprise the chief reasons for the observed clinical and toxicologic effects.

In attempting to make comparisons between human insulin produced by recombinant DNA technology and the situation with asparaginase during its early years of clinical evaluation, one finds more points of divergence than of similarity. While intracellular production in E. coli may be the common source for both proteins, unlike the case with asparaginase, the human insulin will be highly purified at the start of clinical testing. Asparaginase was originally produced by the relatively simple purification techniques of 1967-70 technology. Human insulin will be produced by extensive purification using 1980 technology.

The mode of administration of these two proteins is quite different. Large doses of asparaginase in the order of 200 to 1400 mg per day were administered intravenously to patients over a four to six-week period. The daily dose of asparaginase approximates 100 to 700 times a clinical dose of insulin based on protein. Also, asparaginase has at times been used intermittently in two courses of treatment several months apart, this is not an ideal way to administer a protein with respect to minimizing immunologic complications. Insulin, on the other hand, is generally given continuously in small, daily subcutaneous doses of one to three milligrams over the span of a life-time. Perhaps the most important contrast of all is that E. coli asparaginase is a high molecular weight foreign protein while human insulin is a small, autologous protein.

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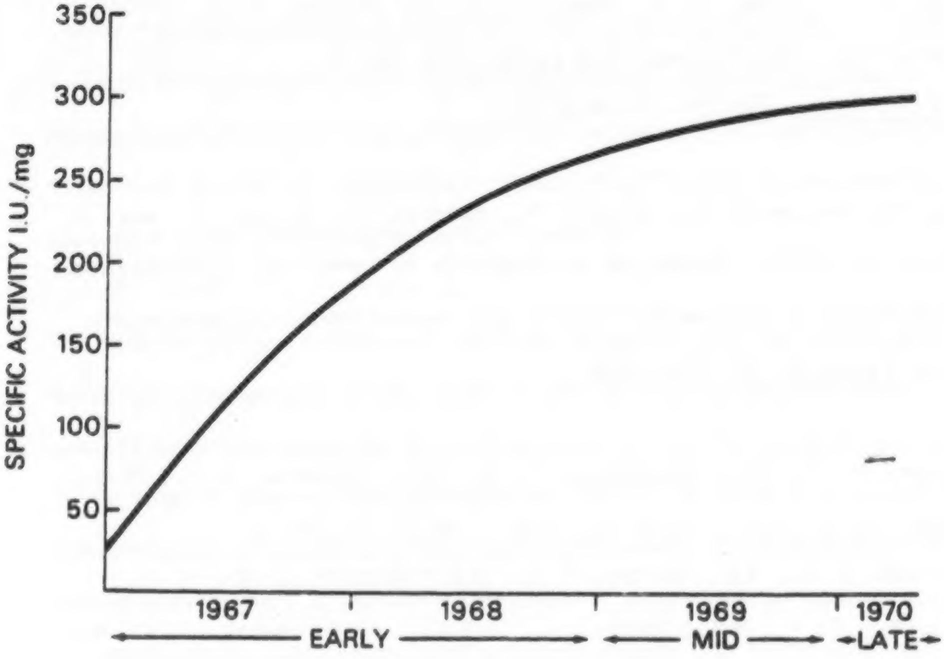

FIGURE 1. L-Asparaginase: Specific Activity of Clinical Trial Lots Versus Time Curve.

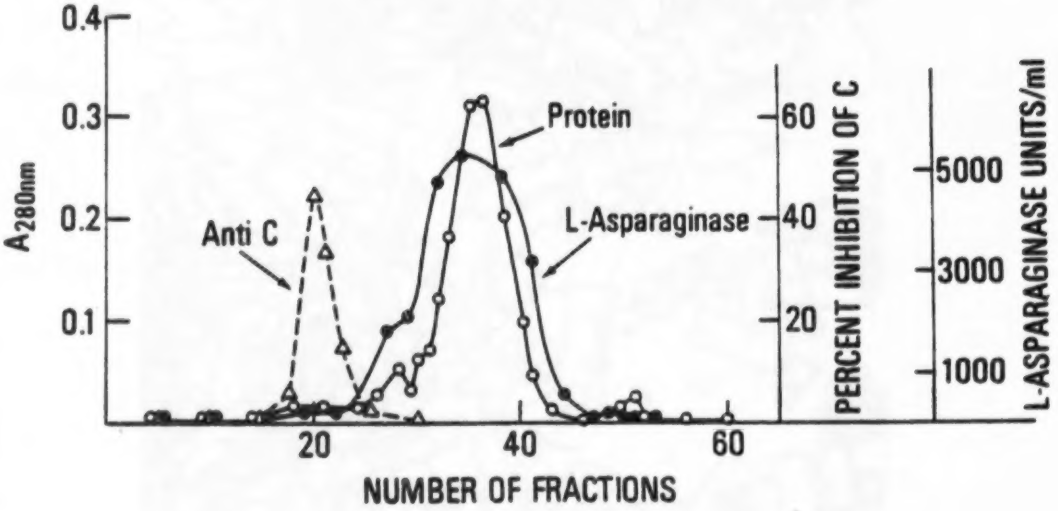

FIGURE 2. Separation of Anticomplementary Activity from L-Asparaginase on Sephadex G-200. (Loos, et al, Cancer Research, 32: 2296 (1972))

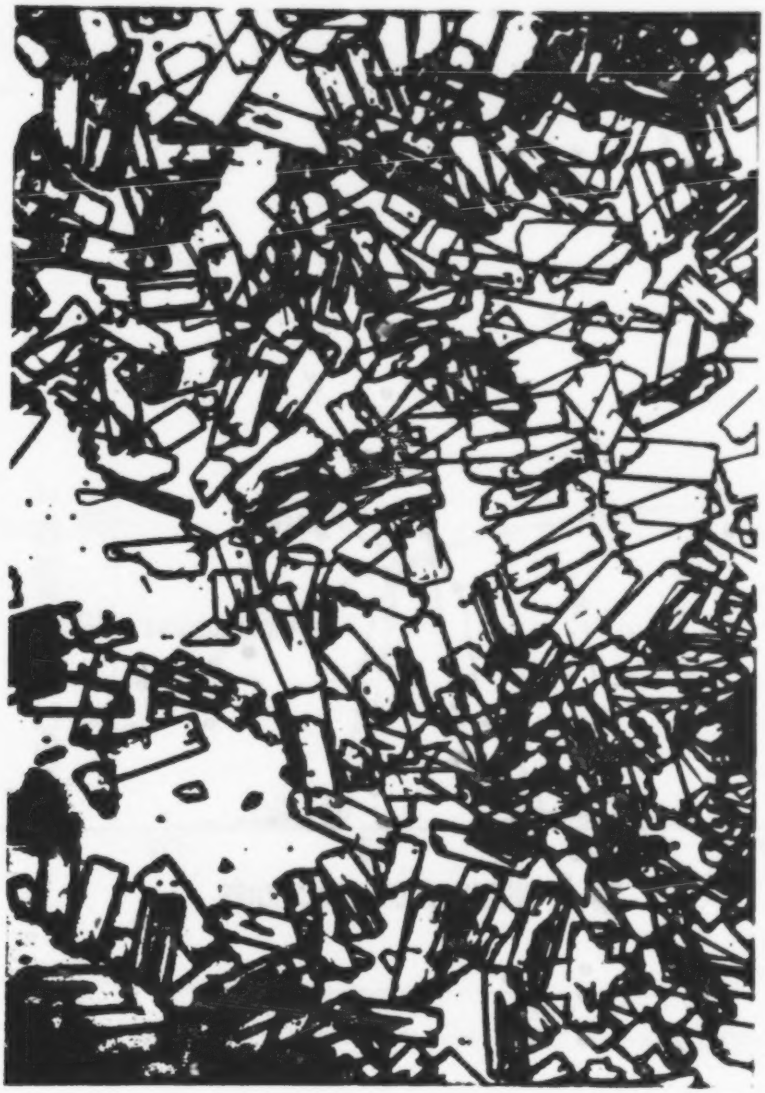

FIGURE 3. Crystalline E coli L-Asparaginase (Lilly), 500X

(Ho, et al, J. Biol. Chem., 245 (14): 3708 (1970))

FIGURE 4. Polyacrylamide Gel Electrophoretic Patterns of L-Asparaginase.

(Ho, et al, J. Biol. Chem., 245 (14): 3708 (1970))

TABLE I

L-ASPARAGINASE - TOXICITY AND SIDE EFFECTS

ENZYME *per se* (Asparaginase/Glutaminase)	BACTERIAL CONTAMINANTS (e.g., Endotoxin)
PANCREATITIS	CHILLS, FEVER
HEPATOTOXICITY (abnormal liver function, fatty liver)	NAUSEA, VOMITING, ANOREXIA
RENAL DYSFUNCTION	HYPERSENSITIVITY (Urticaria, rash, allergy, anaphylactoid reaction)
ABNORMAL CLOTTING	
CNS (Depression, lethargy)	HEPATOTOXICITY?
WEIGHT LOSS	WEIGHT LOSS?
HYPERSENSITIVITY (Urticaria, rash, allergy skin test reactivity, anaphylactoid reaction)	

TABLE 2

L-ASPARAGINASE - CLINICAL TOXICITY

Oettgen, H. F. *et al*, *Cancer*, *25*:253 (1970)

Sloan-Kettering Experience 1967-69

Enzyme Source:	Worthington	19 Lots	35 I.U./mg
	Squibb	16	↓
	Bayer	18	
	Merck	6	360 I.U./mg

Dosage: 200, 1000, 5000 I.U./kg I.V.

Duration: 4 weeks

Patients: 274 (131 children, 143 adults)

Batches accepted for use if pyrogen assay in rabbits showed mean temperature rise of ≤1.5°C in 3 rabbits. Temperatures of this magnitude induced by endotoxin doses 2-3 orders of magnitude below lethal doses. Good correlation between pyrogenicity in rabbits and febrile reactions in patients.

TABLE 3

RESULTS (SLOAN-KETTERING STUDY)

A. Fever. 30% incidence Rough correlation with specific activity (S.A.) of preparations (<70 vs >150 I.U./mg).

B. Anorexia, nausea, vomiting. ~55% incidence rough correlation with S.A. and also dosage.

C. Immune reactions. 30% incidence, first course (41% incidence, second course)

Urticaria	65%
Abdominal cramps	33%
Shortness of breath	22%
Hypotension	18%
Facial edema	11%
Serum sickness syndrome (3 patients)	~ 1%
Antibodies	70%

TABLE 4

L-ASPARAGINASE - BIOLOGICAL TESTS

CT-1570-8A
(132 I.U./mg)

Pyrogens:	Passes U.S.P. requirements at dose of 200 I.U./kg. Mean temperature rise was 0.16°C in five rabbits. 1000 I.U./kg. Mean temperature rise was 0.33°C in three rabbits.
Safety Tests:	4500 I.U./kg injected I.V. in mice caused no deaths over a 7-day observation period.
Depressor Activity:	200 I.U./kg contain less depressor activity than 0.1 microgram histamine base.
Endotoxin:	No evidence of endotoxin by carbon clearance test at i.p. dose of 5000 I.U./kg. (Sensitivity of test: 0.01 microgram/mouse.)

TABLE 5.

PURIFICATION OF L-ASPARAGINASE FROM *E. coli*

Step	Protein[a]	Enzyme Activity	Specific Activity	Yield
	g	I.U. X 10^5	I.U./mg	%
Cell cake (10 kg)		6.5		100
pH 4.5 extract	34.0	5.5	1.6	85
50-90% $(NH_4)_2SO_4$ precipitate	17.3	4.9	28.0	75
Ethanol precipitate	6.0	3.6	60.0	55
Extract of lyophilized powder	4.9	3.5	71.0	54
Crystalline enzyme	0.54	1.6	294.0	25

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[a] Protein of crystalline enzyme was determined by weight after freeze-drying. Protein concentration of other fractions was determined as described under "Experimental Procedure."

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

TO : FOR THE RECORD

DATE: March 6, 1980

Page 39 of Attachment H

FROM : Director
Division of Safety, ORS

SUBJECT: Site Visit, Eli Lilly Co., Large Scale Recombinant DNA Production Facility

On January 28, 1980 the Eli Lilly Co., Indianapolis, Indiana was visited for the purpose of reviewing the facilities, equipment and operations relative to the large scale production of organisms containing recombinant DNA molecules. In addition, discussions were held relative to the draft guidelines governing the large scale production of organisms containing recombinant DNA molecules.

The visit was arranged by Dr. Barkley at the request of Dr. Fredrickson. Dr. Barkley was accompained by two members from the RAC, Dr. Leroy Walters and Mr. Ray Thornton. Dr. Robert W. McKinney, Consultant, Enviro Control, Inc. also participated as a member of the site visit team.

The group representing Eli Lilly was headed by Dr. I. S. Johnson and also included Dr. Lawrence E. Day, Dr. J. Paul Burnett, Dr. David W. Dennen, Dr. Richard H. Baltz, Mr. Max Marsh, and Mr. William B. Young.

Briefing

Eli Lilly has an Institutional Biosafety Committee (IBC) and a Recombinant DNA Protocol Safety Committee. Representation on the IBC includes both company personnel and persons from the community. The Biological Safety Officer is a member of the IBC. It is to be noted that duties of the Safety Officer are collateral to his normally assigned duties. The IBC meets the second Monday of each month and is chaired by Dr. I. S. Johnson.

The Recombinant DNA Protocol Review Subcommittee is comprised of company personnel representing the research staff, corporate safety, and technicians. It is augmented, as required, with other personnel. The Subcommittee meets On Call and is chaired by Mr. M. M. Marsh.

Protocols involving studies with organisms containing recombinant DNA molecules are reviewed by the Subcommittee prior to action by the IBC. Company policy requires that a protocol be submitted for new programs or when there are substantive changes effected in an existing approved program.

The Biological Safety Officer inspects and certifies laboratories to be utilized for the conduct of work involving organisms containing recombinant DNA molecules. The IBC monitors these laboratories by semi-annual inspections.

Personnel engaged in work involving manipulation of organisms containing recombinant DNA molecules are provided medical surveillance. Prior to assignment these personnel are provided a physical examination which includes a variety of clinical procedures. Periodic examination is not provided other than that associated with job associated illness or injury or when an employee has been absent from work for 5 or more days due to non-job associated illness or injury. Personnel absent less than 5 days because of non-job associated illness or injury are not required to report to the medical service prior to re-entry. A physical examination is provided for all personnel on termination of employment.

P3 Laboratories

There are two laboratories (at separate locations) which are utilized for research involving recombinant DNA.

One of the laboratories is a single room which is isolated by an air lock which is fitted with electrically interlocked doors. The laboratory is equipped with a Class II, Type B biological safety cabinet. An autoclave is located in a space which is accessed from the laboratory room. The laboratory is served by an independent ventilation system and is maintained negative to the corridor. Persons entering the laboratory wear protective clothing which is donned and removed in the air lock.

The second laboratory is a complex of rooms, i.e., two biochemistry laboratory rooms, a biological laboratory which serves as the P3 laboratory and a cold room which are served by a common corridor. Access to the area is controlled by key operated doors at each end of the corridor. The ventilation for the area is adjusted so that the corridor is negative to the balance of the building. Internally the biological laboratory is negative to the controlled-access corridor. The biological laboratory is equipped with two Class II, Type A biological safety cabinets. The air balance is monitored through both local and central alarm systems. The exhaust air is passaged through a HEPA filter prior to discharge. The autoclave serving the complex is located on the same floor but is outside the controlled access area. The sinks in the biological laboratory are equipped with foot-operated valves.

Recombinant DNA Production Facility

The facility employed for the conduct of studies for the development of production methods is a single room. Two 150 liter stainless steel fermenters are housed in the room. The room is negative to the surrounding area. Access to this room is controlled.

The fermenters have top mounted drives and all other penetrations are through the top of the vessel. The outgases from the fermenters are treated to effect removal of moisture prior to passage through a particulate sterilizable filter. The agitator shaft penetration is fitted with a leak proof seal. The fermenters are designed to permit withdrawal of samples through a closed system. A variety of sensors attached to each fermenter permit remote monitoring of operating conditions at a central station. Both visual and printout records are available.

The establishment of the integrity of the containment features of the fermenters and the development of certain basic production processes are accomplished with a non-debillitated strain of the host organism to be utilized in production.

Other Facilities

In addition to the foregoing we were provided a tour of large scale facilities that are utilized for the production of antibiotics. In relation to the production of recombinant DNA materials these facilities would probably be suitable for the production of organisms requiring P1 level containment at the laboratory level.

The use of the facilities for P2 level work would probably require some modification to the equipment to accomplish control of aerosols which could be released to the environment through penetrations, e.g., shaft seals, exhaust vents and etc. in the fermentation equipment. For processes which would require manipulation of the viable organism outside the fermenter, a closed system would be required. However, neither the need for modification nor development of closed systems would appear to present a significant problem.

Discussion

Subsequent to the tour of the facilities there was a discussion of the draft (November 14, 1979) guidelines for large scale production of organisms containing recombinant DNA molecules.

In addition to the comments previously provided, Eli Lilly urged that guidelines governing conditions for production of organisms requiring P1 level containment be included. They felt it reasonable to project that production efforts would be accomplished at the lowest permissible level of containment and that modifications to existing equipment, (e.g., elimination of bottom penetrations, control of exhaust gases, sealing of shaft penetrations) would not present a significant problem.

It was also noted that conduct of production at the lowest permissible level of containment would contribute to the development of large scale recombinant DNA production technology by minimizing the need for extensive redesign of existing equipment.

Summary

The visit to Eli Lilly was of real value in gaining an appreciation of the production environment and to accomplish an on-site evaluation of facilities and equipment available for large scale production.

The information and critical comments provided will be of value in the development of the guidelines governing large scale production and in the evaluation of data relative to large scale production programs.

W. Emmett Barkley

W. Emmett Barkley, Ph.D.

U.S. DEPARTMENT OF LABOR
Occupational Safety and Health Administration
WASHINGTON, D.C. 20210

Office of the Assistant Secretary

DEC 17 1978

Donald Fredrickson, M.D.
Director
National Institutes of Health
5600 Fishers Lane
Bethesda, Maryland 20014

Dear ~~Dr.~~ Don Fredrickson:

The Occupational Safety and Health Administration is concerned about potential health hazards to workers exposed to recombinant DNA. This concern is intensified by the increasing commercial uses of recombinant DNA in multiple diverse areas, the prospect of larger quantities being utilized in certain production processes, and the increasing number of individuals who will be exposed as the uses of recombinant DNA materials are further developed.

OSHA supports the program of voluntary compliance with the NIH recombinant DNA guidelines for research and use in areas outside its jurisdiction but feels that more formalized enforcement of procedures and rules within the regulated private sector is warranted. OSHA has a mandate to protect the health and safety of all workers, including workers involved in recombinant DNA research and utilization.

Because the nature of the hazards to individuals working with recombinant DNA remains somewhat theoretical, OSHA strongly urges that a major effort be undertaken to identify and describe the potential hazards to all exposed individuals. Only when the risks are better understood, can a strong enforcement program be undertaken. To help achieve this goal, we would like to recommend that an expert panel under the direction of David P. Rall be convened to specifically address the hazards of recombinant DNA use particularly as they apply to potential occupational exposures. Such a panel would certainly benefit from the active participation by members from the NIH Recombinant DNA Advisory Committee but the panel should also include technical representatives from unions, management, public interest groups, and other concerned parties. OSHA would be happy to help in the selection of the expert panel and would participate as needed.

OSHA would also support an active role by the National Institute for Occupational Safety and Health. A registry of all users of recombinant DNA material would be an important step in defining the scope and extent of use of recombinant DNA material and would facilitate continuing evaluation of any adverse health effects or toxicity. A survey of current users and industrial firms intending to use recombinant DNA and an evaluation of the experience with the current NIH guidelines could provide the basis for a joint NIOSH/ OSHA health hazard alert or a Current Intelligence Bulletin.

OSHA could also review the NIH guidelines and publish them in an appropriate form as interim guidelines to industry. While these would not hold the force of regulations, they would indicate our support for control in this area and would also provide information to employers indicating what we feel are appropriate precautions and procedures of recombinant DNA use.

Finally, the Environmental Protection Agency should be encouraged to explore enforcement of recombinant DNA use through its jurisdiction derived from the Toxic Substance Control Act.

Initiation by OSHA of formalized standard setting is probably premature at the current time primarily because of limited understanding of the potential risks to workers involved with recombinant DNA and the absence of significant practical experience in industrial use. OSHA feels that the above mentioned steps by addressing the potential and real adverse health effects of recombinant DNA would help provide an important basis for future regulatory action.

We look forward to working with you and the Recombinant DNA Advisory Committee on this important public health concern.

Sincerely,

Eula

Eula Bingham
Assistant Secretary
Occupational Safety and Health

cc: Dr. David K. Parkinson
Dr. David Rall
Dr. Anthony Robbins

best copy available

pages tightly bound

WELCOMING REMARKS*

by

Donald S. Fredrickson, M.D.**

I am aware that the RAC has engaged in much discussion of the task it has accepted to review applications involving proprietary information. Usually, these are submitted by private concerns not supported by federal funds and in voluntary compliance with the NIH Guidelines. Particularly, some members of the RAC have chafed under the need for examination and approval of scale-up operations in industry, a result of the 10-liter limit in the Guidelines. Certainly, most members find the task onerous and not a few still find distasteful the scientific review of information which is subject to safeguards including criminal penalties for violation. Some members of the RAC also are of the opinion:

1. that the task involves judgments about apparatus for physical containment which do not rest upon the same experience and training useful for decisions about molecular biology and other aspects of recombinant technology; or
2. that when the judgments about physical containment require site inspections in industry, such visits belie the insistence of NIH, and its Director, that this agency eschews a regulatory role; and several members believe
3. consideration of any part of an application from the private sector, perhaps even any proprietary data, should be done only by a regulatory agency, which NIH is not.

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* Presented at the meeting of the Recombinant DNA Advisory Committee (RAC) at NIH on June 5, 1980.

** Director, National Institutes of Health, Bethesda, MD.

I am grateful to all the members of the RAC, whatever their views on this subject, for subordinating their feelings to the extent that they have continued to carry out these important duties. In so doing, they have made it possible for all users of recombinant technology in the U.S.A. to do so under two important conditions that obtain in nearly all other advanced countries of the world. These are:

(1) the determination of appropriate procedures by a single national body, responsible for interpreting and developing uniform national guidelines, and

(2) the regulation of a rapidly evolving science without passage of special statutes, avoiding inflexibility often attendant upon such legislation.

These important conditions are among those necessary for maintenance of parity among the nations for safe use of this technology and access to its benefits. It is essential that the RAC, whose deliberations affect the course of this field of science throughout so much of the world, not alter these conditions by precipitous changes in its procedures.

Nevertheless, I am sympathetic to this malaise of the RAC. We must try to relieve it--and to move NIH back from what some see as a position on the brink of regulatory involvement. In thinking about such relief, I note especially the rising interests of OSHA and NIOSH in ascertaining any threats to workers inherent in industrial scale-ups. FDA and other regulatory agencies have also begun to test their reflexes in a similar way. FDA sponsored here this week a useful meeting on industrial application of recombination for production of biologics.

It is appropriate that these agencies begin to consider their mandates as industrial use of recombinant techniques grows. One of the earliest tasks of the Federal Interagency Advisory Committee on Recombinant DNA Research (IAC) was an analysis of the authorities of the regulatory agencies in this area. The results indicated that no single agency had clear authority over laboratory research, but there obviously were appropriate roles for such agencies in the oversight and regulation of industrial-scale use of recombinant methodologies. All the regulatory agencies also have continuously been kept fully informed of the activities concerning the NIH Guidelines, through their membership on the IAC, and through their liaison membership on the RAC.

At the same time, it should be stated here with candor that no federal regulatory agency presently has anything like the in-house competence to perform--for industrial applications--all of the present tasks of the RAC. Development of such competence will require time and expense, and it would not arise suddenly upon the passage of any statute. Furthermore, we are still at a stage where the creation of more than one RAC in this country--one for industry, one for non-profit research--would be inefficient and destroy the unity of information-sharing and decision-making about the many aspects of handling recombinant DNA activities that facilitate appropriate evolution of guidelines.

It is, however, apparent that in taking any actions appearing to validate physical containment on an industrial scale, the NIH also has insufficient in-house competence, and, like the RAC, must rely on consultants. In these determinations, a regulatory agency like OSHA, or its researcher partner, NIOSH, can use consultants as effectively as can NIH, and may have more staff competence.

Such judgments can more appropriately be carried out by them, especially when they are related to a regulatory mission.

From these considerations, I believe that the RAC should consider at least two options as it deliberates upon its procedures for handling industrial or private requests:

(1) to continue as it is until more experience dictates some new mode. Here keep in mind that an Industrial Practices Subcommittee of the IAC is actively studying the problem and will report to the IAC in the fall.

(2) to restrict its review and decisions to questions concerning biology and related issues of laboratory containment; and when physical containment for large-scale use is the issue, to judge only whether the specified P-LS level is appropriate (referring to the model designations in the April 11, 1980, Federal Register). The regulatory agencies will be fully apprised of such actions. They can make on-site evaluations if they deem them to be appropriate to their regulatory mandate.

With regard to the second option, I would note here that past actions of the RAC have included the insertion of a provision for visits by an NIH-designated person to industrial facilities that have been the subject of a RAC decision. If such visits are for purposes of correlating scientific information relevant to the duties of the RAC, they would seem to me to be still appropriate. Inspections for regulatory purposes shall be carried out by OSHA/NIOSH or other regulatory agencies having relevant authority.

Finally, let me emphasize that it would not be appropriate for the RAC to decide, in exasperation, that it would like to avoid handling any proprietary data. From time-to-time, such material will have to be evaluated by the RAC in pursuit of its duties.

I welcome the opportunity to participate in discussion of this matter to the extent that my presence will be useful.

INDUSTRIAL PRACTICES SUBCOMMITTEE OF THE FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

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INDUSTRIAL PRACTICES SUBCOMMITTEE OF THE FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

Minutes of the Third Meeting*
Thursday, September 4, 1980
1:00 to 3:00 p.m.
Room 3104, New Executive Office Building
Washington, D.C.

Chairman's Introduction

Dr. Omenn stated that he is impressed with how much the Subcommittee has gotten into the substance of developments in industrial applications of recombinant DNA techniques.

A number of Subcommittee members said they had been contacted by staff of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. (Executive Secretary's note: The Commission discussed at their July 11-12 meeting, and will discuss again at their September 15-16 meeting, a message from the general secretaries of the National Council of Churches, the Synagogue Council of America, and the United States Catholic Conference stating that for "genetic engineering...we believe...that no government agency or committee is currently exercising adequate oversight or control, nor addressing the fundamental ethical questions in a major way." A July 17 letter from the Commission Chairman, Morris Abram, to President Carter says the Commission "decided to survey the field to determine whether there is a need for us to address any ethical issues raised by genetic engineering.") Dr. Omenn said that his advice to the Commission has been to educate themselves on developments in this field, including the considerable body of published literature, and that, in his opinion, they should rank other issues as higher priority for their attention at present. Dr. Omenn described as unfortunate and inaccurate the accusation that Federal officials and scientists involved in this field think only of their narrow interests, and do not consider the wider ramifications of societal debate involving the nature of man and societal responsibilities.

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* A list of attendees is appended as Attachment A.

Report from OTA

Dr. Harsanyi said that the OTA report "Impacts of Applied Genetics," originally due to be completed in August 1980, is now slated for submission to the Technology Assessment Board about December 1, 1980. If approved by the Board, it will be released to the public about January 15, 1981. Dr. Robbins requested that the part of the report giving an estimate of the future magnitude of industrial applications of genetic engineering (capital, workers, etc.) be released to the Industrial Practices Subcommittee earlier than January 1981. Dr. Omenn asked Dr. Harsanyi to take this request under consideration.

Report form NIOSH

Dr. Robbins distributed the report of the NIOSH walk-through survey, conducted on March 28 at the Eli Lilly and Company Research Laboratories, Indianapolis, Indiana. (The report is appended as Attachment B.) Mr. Elliott reported that Lilly was pleased with the report and that, in response to an invitation from Lilly, NIOSH may return for a more in-depth industrial hygiene survey. Dr. Robbins said that NIOSH walk-through surveys have also been conducted at Genentech and Cetus. The final reports of these surveys are not yet available, as it is NIOSH policy to first send a draft report to the group visited and to consider modification of the report based on comments received before finalization. When finalized, the reports will be shared with the Industrial Practices Subcommittee.

The team that went on the walk-through surveys is the nucleus of a group of NIOSH biological scientists, industrial hygienists, chemists and engineers who will look at occupational health issues involved in industrial uses of recombinant DNA. Continued industrial hygiene surveys will be done for fact-finding to see if problems exist. These are routine components of NIOSH research investigations at industrial work sites. Dr. Omenn expressed the hope that the current cooperation between NIOSH and industry would continue and not develop into an adversary situation.

NIOSH and EPA are considering joint funding, by contract, of assessments of the adequacy of currently used "control technology." The question of what is adequate and reasonable medical surveillance is a problem in general in occupational medicine. A group at NIOSH working with CDC and outside consultants is developing general draft recommendations. They may use recombinant DNA as a test case.

NIOSH is looking at the adequacy of worker education in general, attempting to come up with recommendations of reasonable approaches. Dr. Omenn endorsed this effort, noting that reports in the media may lead to worker anxiety; it is good for workers to know what they are working with and not working with. He also mentioned the "second-year medical student syndrome" and therefore the importance of objective rather than subjective measures of effects.

Report from EPA

Dr. Levin reported that two EPA workshops have been held: one on sewage, water, and health effects problems at the University of Rhode Island in October 1978; and one on escape, containment, and ecological problems at the University of California, San Diego, in November, 1980.

An in-house group is evaluating existing legal and regulatory mechanisms available to EPA in regard to the applied genetics industry. Exploratory efforts have been initiated to evaluate beneficial uses of the products of this industry in reducing existing hazards to the environment.

An in-house group, together with investigators at Tufts University and the University of Rhode Island, will soon begin evaluating the biological containment of EK1 host cells and vectors during sewage treatment. Two contracts are about to be let on Assessment of Future Environmental Problems: Applied Genetics. One deals with agricultural concerns, the other with industrial concerns.

Grants will be awarded for A Probability Analysis of Escape and Establishment of Laboratory Organisms (this will involve modeling and fault-tree analysis); and to study The Fate of Novel Genomes in Established Ecosystems (tracing the survival of labelled organisms). Dr. Harsanyi suggested that it might be useful to ascertain the survival of organisms currently used for pollution control. Dr. Still suggested the same for legume innocula microorganisms used to encourage biological nitrogen fixation.

Report from FDA

Dr. Goldberg noted that FDA has regulatory authority concerning only the products of recombinant DNA techniques, as distinct from other agencies whose concerns are primarily with the process. Five of the six FDA Bureaus will probably be dealing with products made by recombinant DNA techniques. The Bureau of Drugs will deal with pharmaceuticals, such as insulin and growth hormone. Single-cell protein would be evaluated by the Bureau of Foods. Human vaccines or interferon would be considered by the Bureau of Biologics. Animal drugs would be reviewed by the Bureau of Veterinary Medicine. Enzymes that are part of an *in vitro* diagnostic system would be considered by the Bureau of Medical Devices. Since the different FDA Bureaus have different statutory bases, each product of recombinant DNA techniques will be dealt with individually by the relevant FDA Bureau.

Most insulin, as made today from calf or pig pancreas, is only about 80-90 percent pure. Identity and purity are checked by relatively crude analytical techniques. When checking insulin made in bacteria by recombinant DNA techniques, more sophisticated tests for identity and purity, such as high-pressure liquid chromatography and amino acid sequence, will probably be used. Contaminants in this case will be different, including bacterial polypeptides and polysaccharides, and different tests for antigenicity and pyrogenicity will probably be required. Higher standards will probably be set, leading to higher purity of the product.

Dr. Goldberg said FDA inspectors are primarily concerned with contamination of the product. However, according to the inspection referral system instituted by the Interagency Regulatory Liaison Group, he expects that an FDA inspector noting a possible violation in the regulatory domain of another agency, such as OSHA or EPA, would make the appropriate referral.

Report from USDA

Dr. Still reported that to vaccinate against Foot and Mouth Disease an attenuated virus is used at present, which in a certain percent of cases actually causes the disease. If current attempts to use recombinant DNA techniques to produce a vaccine, involving only viral coat proteins with no live virus, are successful, this would be a major advance in control of this economically important disease.

Other uses of recombinant DNA techniques of importance to agriculture are highly speculative. These include:

- production in bacteria of agriculturally and economically important materials such as plant growth regulators, natural products with insecticidal properties or amino acids, vitamins, etc., required for the nutrition and sustenance of man;

- changes in the germ plasm of plants to alter protein storage proteins, to enhance plant resistance to microbes, insects, nematodes, or stress, or to introduce the ability to fix nitrogen;
- changes in biological control agents to act against insect predators.

No health hazard to man in any of these activities has been identified at this time.

Report from Department of Commerce

Dr. Gordon distributed copies of his prepared remarks comparing the hazards of making the same product by different methods. (This is appended as Attachment C.) He considered the production from ethylene of ethyl alcohol, ethylene oxide, and ethylene glycol, comparing the current petrochemical operations with possible recombinant DNA procedures. Current procedures use high temperatures (up to 400°C) and high pressures (up to 1000 psig). At such extreme temperatures and pressures, failure of reactor vessels or lines would indeed be catastrophic. The chemical industry has a very low accident rate for this type of operation, but with recombinant DNA techniques, because they use less extreme conditions, catastrophic accidents are even less likely.

Next Subcommittee Meeting

Dr. Omenn indicated that he will be sending Dr. Fredrickson, Chairman of the parent Committee, an interim report on the activities of the Industrial Practices Subcommittee, appending copies of the minutes of the three Subcommittee meetings. (Executive Secretary's note: Dr. Omenn's memorandum to Dr. Fredrickson is appended as Attachment D.)

Dr. Omenn suggested the next Subcommittee meeting be held towards the end of the year. (Executive Secretary's note: The next meeting has been scheduled for Tuesday, December 16.)

Bernard Talbot

Bernard Talbot, M.D., Ph.D.
Subcommittee Executive Secretary
September 23, 1980

LIST OF ATTENDEES

Meeting of September 4, 1980

CHAIRMAN

Dr. Gilbert S. Omenn
Associate Director for Human Resources, Veterans, and Labor
Office of Management and Budget

EXECUTIVE SECRETARY

Dr. Bernard Talbot
Special Assistant to the Director, NIH

DEPARTMENT OF AGRICULTURE

Dr. Gerald Still
Science and Education Administration

Dr. Sue Tolin
Science and Education Administration

DEPARTMENT OF COMMERCE

Dr. George Gordon
Office of Environmental Affairs

DEPARTMENT OF HEALTH AND HUMAN SERVICES

OFFICE OF THE ASSISTANT SECRETARY FOR HEALTH

Dr. Lowell T. Harmison
Science Advisor, Office of Environmental Affairs

Mr. Philip Janus
Staff Assistant, Office of Environmental Affairs

CENTER FOR DISEASE CONTROL

Dr. John H. Richardson
Director, Office of Biosafety

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH, CDC

Dr. Anthony Robbins
Director, NIOSH

Mr. Seth Pauker
Health Scientist, Office of Program Planning and Evaluation

Mr. Larry Elliott
Industrial Hygienist
Division of Surveillance, Hazard Evaluation, and Field Studies

NATIONAL INSTITUTES OF HEALTH

Dr. Robert McKinney
Chief, Occupational Safety and Health Branch
Division of Safety

FOOD & DRUG ADMINISTRATION

Dr. Michael Goldberg
Special Assistant to the Director, FDA

Dr. Timothy J. Henry
Staff Science Advisor

DEPARTMENT OF LABOR

Dr. Lucile Adamson
Consultant, Division of Health Standards, OSHA

Dr. Chia T. Chen
Senior Industrial Hygienist
Office of Technical Support, OSHA

ENVIRONMENTAL PROTECTION AGENCY

Dr. Morris Levin
Director, Innovative Research Program

Dr. Richard Hill
Acting Director, Health Effects and Science Policy

NATIONAL SCIENCE FOUNDATION

Dr. Herman Lewis
Head, Section on Cellular Biology
Division of Physiology, Cellular and Molecular Biology

EXECUTIVE OFFICE OF THE PRESIDENT

OFFICE OF SCIENCE AND TECHNOLOGY POLICY

Dr. Richard Adamson
Senior Policy Analyst

OFFICE OF TECHNOLOGY ASSESSMENT

Dr. Zsolt Harsanyi
Project Director, Genetics

Walk Through Survey Report
of
Eli Lilly & Company Research Labs
Indianapolis, Indiana

Survey Date:
March 28, 1980

Report Written By:
Larry Elliott

Survey Team:
George Carson
Larry Elliott
Seth Pauker
Dave West

Date of Report:
June 26, 1980

Industrial Hygiene Section
Industry-wide Studies Branch
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health
Cincinnati, Ohio

PLACE VISITED:	Eli Lilly & Company Research Laboratories Indianapolis, Indiana
DATE OF VISIT:	March 28, 1980
PERSONS(S) CONDUCTING SURVEY:	George Carson Larry Elliott Seth Pauker David West
EMPLOYER REPRESENTATIVE:	Irving Johnson, V.P. of Research (317) 261-4391
UNION:	None
STANDARD INDUSTRIAL CLASSIFICATION OF PLANT:	2834 - Pharmaceutical Preparations
PURPOSE OF SURVEY:	To investigate and evaluate Eli Lilly's methods of containment, environmental monitoring, and health surveillance for production/research processes utilizing recombinant DNA techniques.

ABSTRACT

A walk through industrial hygiene survey was conducted at the Eli Lilly and Company Research Laboratories in Indianapolis, Indiana on March 28, 1980. This company is involved in research and development of recombinant DNA fermentation processes applicable to pharmaceutical preparations. Assessments of control technology, including containment design, validation procedures, and work practices were made during this visit. Recommendations for a worker registry, preventive maintenance program, and controls to minimize potential hazards to the worker are included.

INTRODUCTION

The public debate over genetic engineering has focused on the possible hazards of genetically modified microorganisms, potential health hazards to workers involved with industrial application of recombinant DNA techniques, and the utilitarian prospects of such technology. Several risks assessment programs designed to investigate some of the characteristics of proposed host vector systems which might affect hazard potential have been conducted by interested scientists. Likewise the benefits from recombinant DNA technology are being as vigorously promoted. The National Institute for Occupational Safety and Health (NIOSH) is responsible for conducting research, recommending occupational safety and health standards, providing technical assistance to workers and employers, and conducting training and educational programs. NIOSH's responsibility extends to both existing and emerging technology which might impact on worker health and safety. Thus, NIOSH is evaluating the potential occupational hazards involved with recombinant DNA technology.

This research effort was prompted by the anticipated surge of recombinant DNA techniques in various industrial processes. Genetic engineering technology may be utilized in various manufacturing processes in the areas of agriculture, organic chemicals, energy, food processing, and pharmaceuticals. This potential growth and the possibility of uncharacterized occupational exposures indicate the necessity for careful evaluation of health risks. NIOSH is accustomed to examining new technologies for potential occupational hazards and developing recommendations for safeguarding the workers health. Implementation of safeguards and protective engineering controls early in the growth of an industry can only minimize human suffering and avoid expensive retrofitting of production systems.

Under the Occupational Safety and Health Act of 1970, Public Law 91-596, the National Institute for Occupational Safety and Health (NIOSH) was mandated and authorized to conduct research and health studies. Specifically, Section 20(a)7 states that NIOSH shall conduct and publish industrywide studies of the effect of chronic or low level exposure to industrial materials, processes, and stresses on the potential for illness, disease, or loss of functional capacity. Thus, this research is pursuant to the development of health standards applicable to a broad range of occupational environments. In compliance with this mandate, the Industry-wide Studies Branch of the Division of Surveillance, Hazard Evaluations and Field Studies is conducting a study to assess the potential occupational hazards in research and the commercial application of recombinant DNA technology.

In order to analyze the current technology for potential occupational hazards, NIOSH plans to conduct several walk through surveys of recombinant DNA processes and associated industries. Identification of potential hazards, with recommendations to minimize worker exposure, will be derived from the information gained during these surveys. This report contains recommendations relevant to operations at Eli Lilly and Company. Recommendations applicable to the entire industry employing recombinant DNA technology will be made after NIOSH completes a thorough assessment. An assessment of the following areas will be made:

- process operations with attendant potentials for worker exposure
- engineering controls (e.g. physical containment design, ventilation, exhaust gas filtration, waste product controls, etc.)
- validation procedures (e.g. of sterilization, physical containment, and process termination)
- work practices
- emergency and accident procedures
- medical surveillance
- environmental monitoring
- numbers of exposed workers
- employee training and education

This survey of Eli Lilly and Company in Indianapolis was conducted as one of a series of initial walk through surveys of firms utilizing recombinant DNA techniques in research or production processes.

DESCRIPTION OF THE FACILITY

Eli Lilly and Company, with multiple manufacturing sites in the United States and abroad, has its headquarters and many of its research and manufacturing operations in Indianapolis, Indiana. The company was founded in 1876 in downtown Indianapolis. Divisions of pharmaceuticals, agricultural products, cosmetics, electronic medical instruments, cardiac pacemakers, and research make up the corporate structure.

Eli Lilly's vast experience in the fermentation industry recently has been applied to the research and development of recombinant DNA technology. This is exemplified by the sophisticated equipment and systems used in Lilly's recombinant DNA processes. Eli Lilly is conducting its research and developing production under voluntary compliance with the National Institutes of Health (NIH) Guidelines for Recombinant DNA Research.

At the time of this survey, genetic engineering research was conducted in building 28, 731 S. Alabama St. and building 328, 1200 Kentucky Avenue. The main production development efforts are ongoing at the 1200 Kentucky Avenue location which houses various P-2, P-3, and P-1 containment level laboratories.

DESCRIPTION OF THE WORKPLACE

The Indianapolis facilities employ approximately 6,000 persons of whom about 4,200 are engaged in direct production of pharmaceutical products.

Since recombinant DNA fermentation is in the research/production development stage, very few employees are currently involved. The anticipated number of workers involved with recombinant DNA fermentation processes is expected to be minimal due to the nature of the process and the manpower requirements dictated by the system technology.

DESCRIPTION OF THE PROCESS

Fermentation processes, including those involving recombinant DNA, start in a P-2 or P-3 lab setting as ampoules of culture stored in N_2 which are then used to inoculate a shake flask containing the appropriate nutrients. After an incubation period the inoculum (the contents of a shake flask) is transferred, in a specially designed metal container, to the production floor. This container is designed to preclude escape or entry of viable organisms during inoculation of the fermentation vessel. Metal fermentation vessels used by Lilly for recombinant DNA range in sizes from 10-liters, 150-liters, or 2000-liters, depending upon the process, stage of development, or stage of culture growth. Culture is introduced into the 150-liter vessel or the 2000-liter vessel, by a stainless steel vessel that is sterilizable after transfer of the contents and before the vessel is detached from the fermentator, the vessels are charged with nutrients, water, and inoculum by closed system design to maintain physical containment.

The fermentation process is monitored and is terminated by chemical or physical means when the harvest stage is attained. Eli Lilly is currently using thermal techniques to terminate the process. The batch is then removed from the fermentor by pressure, and extraction of the desired product takes place. The mechanism of extraction may be by centrifugation or lysogenesis, depending upon the nature of the process and the desired product. Product extraction operations were not viewed during this visit.

The fermentor, inoculating device, shake flask and all exposed equipment are sterilized with steam after use to prevent contamination of succeeding operations and work environment.

DESCRIPTION OF INDUSTRIAL HYGIENE, SAFETY, AND MEDICAL PROGRAMS

Safety and Health

The company has a well organized safety and health program that is steered by the Corporate Safety and Health Committee and the Department of Industrial Health and Safety. Safety representatives are located at each site to monitor the work practices of employees. Periodic safety and housekeeping inspections are made in each plant by the plant safety representatives and a member of line supervision. Departmental and divisional safety committees, with rotating memberships, meet monthly for review of safety and/or health issues and to make recommendations.

The mayor of Indianapolis, a vice president of Indiana University (who is a physician), and a pathologist from Indiana University Medical School (an expert on infectious disease) are the non-company members of the IBC. The IBC reviews the work of the protocol review subcommittee (of the corporate safety and health committee) which is responsible for making containment recommendations for all recombinant DNA protocols, laboratories, production areas and procedures.

Work practice guidelines are maintained and available for specific procedures requiring personal protective equipment or precautions. Specialized safety equipment, laboratory coats, shoes, and glasses are provided by the company. Specific training programs for employees involved with recombinant DNA procedures have been instituted to instruct them in good work practices, process requirements, and emergency procedures. These include audio-visual presentations and instruction by supervisors. Communication meetings are held to discuss the status of recombinant DNA technology, the NIH Guidelines, and Lilly's advances in the field.

Industrial Hygiene

Environmental monitoring is limited to RODAC (Replicate Organism Direct Agar Contact) plates, air impaction onto agar plates, settling plates filled with agar, and liquid impinger techniques. Monitoring is performed on a scheduled basis during each run, and records are maintained. No viable recombinant organisms have been recovered. However, the sensitivity and accuracy of sampling and analytical techniques have not been documented for various culture and environmental conditions which may be encountered; the ability to recover viable culture organisms from contaminated surfaces or air has not been determined.

Medical Program

The company's medical department is staffed by a director of industrial medicine, five full-time physicians, and eight nurses who serve the employees in the Indianapolis area. Pre-employment physicals are given to newly hired employees. Comprehensive annual physicals are given to all employees and annual physical examinations are mandatory for all laboratory and production personnel (including recombinant DNA workers). Company policy states that all injuries must be reported to the plant physician and any employee on sick leave for more than five days must report to the physician before returning to work. Employees undergoing antibiotic therapy are excluded from working in recombinant DNA areas. Employees involved with recombinant DNA processes who develop chronic or acute illness are required to see the physician. Preassignment blood serum samples are maintained for individuals working with recombinant DNA processes. When appropriate, immunizations are given to those employees working with infectious organisms.

DESCRIPTION OF PAST EXPOSURES

Data on exposure of employees during fermentation processes with organisms containing recombinant DNA are limited to the results of the environmental monitoring previously mentioned and the medical surveillance program Eli Lilly has implemented. No quantitive data are available to document range or incidence of exposure. The company voiced concern about the practicability of the methods currently used for environmental monitoring, yet recognized the need for such monitoring.

EVALUATION OF THE PLANT AND PROGRAMS

Laboratory and Production Facility Design

Physical containment laboratories at the P-2 and P-3 level of containment used for research and development of recombinant DNA techniques were toured. These were well designed and were basically operated within the NIH Guidelines for Recombinant DNA research. A P-3 level laboratory utilized for research and shake flask fermentation was toured; no apparent problem areas were noted. A P-2 laboratory with 10-liter fermentors and benchtop continuous culture apparatus (being readied for operation) housed work concerning laboratory-to-production development.

The equipment specially designed for large scale growth of recombinant DNA organisms was housed in two locations. A room, separated by a partition from a conventional pilot fermentation area, housed modified 150-liter fermentors. A P1-LS (large scale) area with a 2000-liter fermentor, modified to contain recombinant DNA organisms will be used for production development and production of clinical trial material. This fermentation system was still in the developmental phase, and undergoing sterilization validation procedures, at the time of this survey; fermentation with recombinant DNA work is not housed in a distinctly confined room, but is in a pilot production area in immediate proximity to conventional fermentors of the same size. Access to the tops of the fermentors is by a single stairway and catwalk. Descriptions of fermentor modifications may be found in the following section and appendix 1 of this report.

Engineering Controls

As with other fermentation processes the concept of containment is not only to prohibit escape of organisms from, but also to exclude entry into the system. Thus, the engineering design and measures implemented by Eli Lilly to achieve the dual aspect of containment are quite sophisticated. This attention to process integrity and thus, effective control for personnel exposure demonstrates the level of containment that Eli Lilly attempts to maintain with recombinant DNA fermentation processes. Because of the design of this large scale operation, it is possible that more risk for worker exposure to recombinant DNA organisms (e.g. from accidental spillage or aerosol production) would occur in the shake flask laboratory than on the production floor.

The ventilation systems, exhaust gas filtration, waste products control, biological safety cabinets, barriers to unrestricted access, and all interior room surfaces in the P-3 and P-2 laboratories conformed to the NIH Guidelines. The NIH Guidelines for the P1-LS level does not require specific ventilation measures, or a distinctly confined area. Characteristics of fermentors used by Eli Lilly for organisms containing recombinant DNA molecules are:

1. Inoculation, sample collection, nutrient addition, and transfer of culture fluids can be performed with closed system piping. All pipes and feeder tubes are connected to a fermentor at its top.
2. Each fermentor has its own separate system of feeder lines, with the exception of electrical service, steam, and pH control (the latter two of which are double-check valved).
3. Agitator shafts which penetrate the fermentor shell have double mechanical seals and are top mounted on the fermentor.
4. The 150-liter fermentors are operated under slightly negative pressure, but the 2000-liter fermentors are operated at a slightly positive pressure (approximately 5 psi).
5. Sterilization of fermentor exhaust is accomplished by double filtration.
6. Drainage pipes at the bottom of fermentors are sealed closed.
7. Before initial use, the ability to sterilize an entire fermentor and its ancillary piping is verified by a test protocol employing thermocouples.

Validation Procedures

Validation of thorough sterilization of the fermentor, inoculation device, and exhaust gases is the responsibility of a validation team. A design engineer, two systems technicians, and the lead operator make up the team. Thermocouples and a recording system is utilized to assure that all points of the fermentor have been effectively sterilized. Each team member holds equal responsibility in giving approval for production start up of the fermentor. Thus, each must be confident that containment, sterilization, and inactivation procedures and systems are effective for size of the system, the specific host organism, and the recombinant DNA molecule.

The validation team also reviews the work procedures and practices of the operator during the fermentation cycle. Recommendations and changes in procedure can then be made by the team toward the prevention of breaching the containment design (resulting in exposure).

All biological safety cabinets undergo scheduled certification to demonstrate adequate performance. Autoclaves are also monitored for performance by use of recording charts. Centrifuge equipment received periodic maintenance checks to assure aerosol production is contained. Controlled access to the P-3 level laboratory is practiced and monitored by the laboratory supervisor. The laboratory ventilation systems are equipped with alarms that actuate in the event of a system malfunction.

Work Practices

No unsafe practices or poor laboratory techniques were noticed during this survey. The validation team line operators, supervisors, and the Corporate Safety and Health Committee monitor for poor personal hygiene habits and work practices. Adherence to safe work practices and company rules is required by the company. Maintenance of the fermentation systems and in the laboratories is performed only after lock out procedures have been implemented and approval granted from the line operators and the line supervisor.

Emergency and Accident Procedures

Plans for emergencies and laboratory accidents outline specific procedures for accidental spills in the laboratories. Corrective action for failure of equipment and/or facility safeguards are also included. Plans also exist for fire, explosion, and natural disaster. All of these plans are established and well known to those who work in the areas covered by the plans.

CONCLUSIONS

Experience has demonstrated that safe conduct of research and production processes involving potentially hazardous entities is dependent on good work practices, availability and use of effective control technology, and effective management. Advancement of recombinant DNA techniques into production processes is being undertaken at Eli Lilly in a responsible manner employing all of Eli Lilly's relevant expertise.

Evaluations of the product extraction process should be conducted once the company has developed the process to the point of utilization.

RECOMMENDATIONS

The PI-LS (2000-liter) production area should be segregated from other production operations by structural confinement. Additional containment controls could be implemented dependent upon future processes, host organisms, recombinant DNA molecules, and product of interest. This will maintain the integrity of the process and procedures outlined for recombinant DNA fermentation processes. Such system confinement will also enhance the containment of the entire operation to preclude accidental exposure to other employees and processes. This should be within the interests of good manufacturing procedures of the pharmaceutical industry.

A scheduled systematic preventive maintenance program for agitator seals, control valve, pressure release valves, and equipment/facility safeguards should be implemented. Possibly the validation team (with a maintenance representative) could manage such a program.

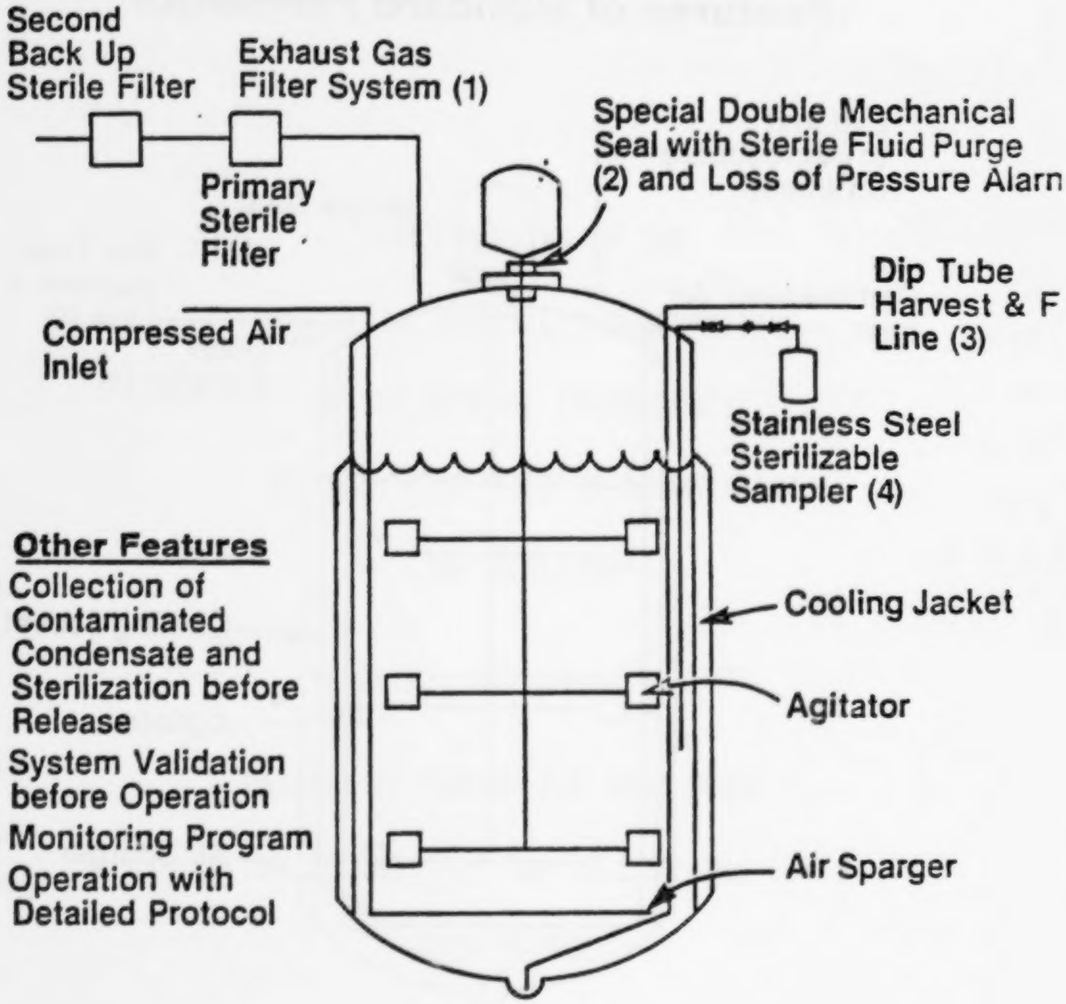
The efficacy, sensitivity, and accuracy of environmental sampling and analytical techniques should be investigated. A sampling protocol documenting procedures, analysis, sampling points, and sampling schedule requirements should also be devised.

An observation was made of a chemostat, with temporary tubing and fittings, sitting on an open laboratory table top in a P-2 laboratory. It is recommended that all chemostats, operating with recombinant DNA molecules or infectious organisms, be set up in class 1 biological safety cabinets or with secure leak proof tubing and fittings.

A registry of all owrkers employed with recombinant DNA processes should be established and maintained for reference to determine any abnormal medical problems or unusual occurances that someday may be attributed to recombinant DNA processes. NIOSH has had considerable experience in designing and maintaining such worker registries and can thus, provide assistance in design, implementation, and logistical requirements. The comparability of individual company registries is desirable for optimum and successful surveillance on an industry wide basis.

APPENDIX 1

Features of Contained Fermentor
Eli Lilly 2,000 Liter Fermentor

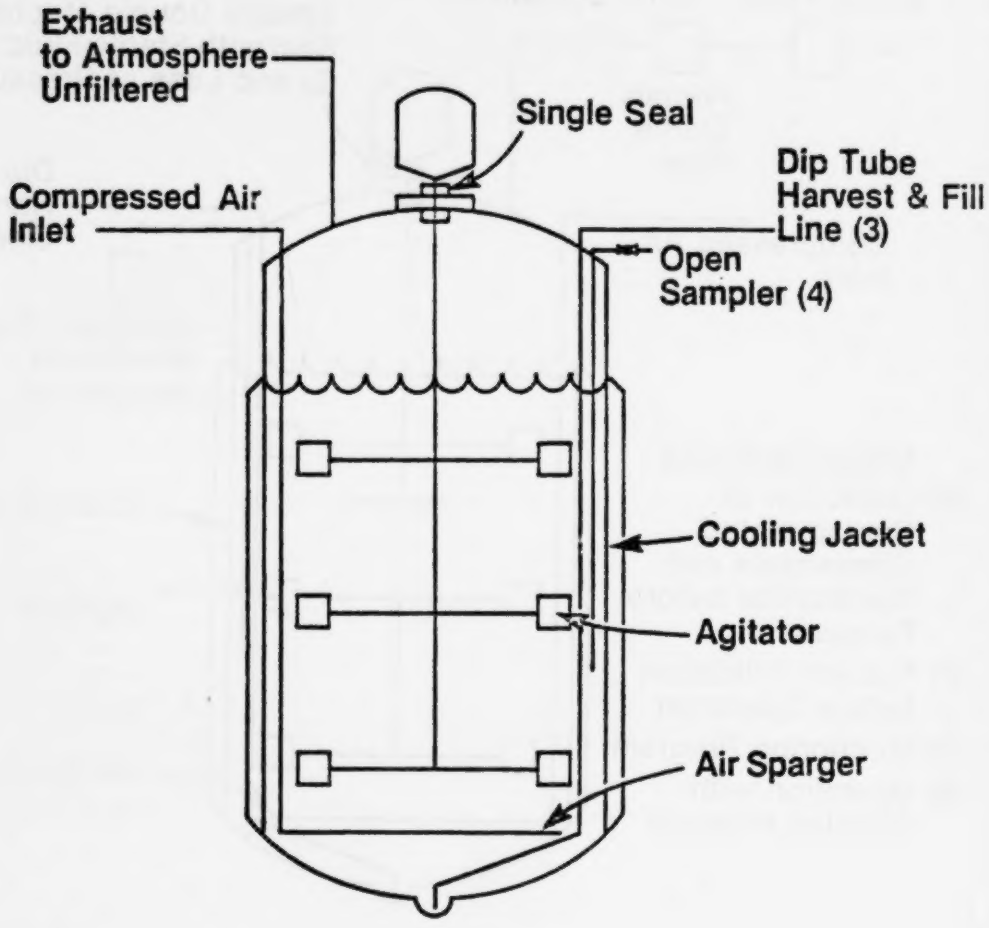

Second Back Up Sterile Filter
Exhaust Gas Filter System (1)
Primary Sterile Filter
Special Double Mechanical Seal with Sterile Fluid Purge (2) and Loss of Pressure Alarn
Dip Tube Harvest & F Line (3)
Compressed Air Inlet
Stainless Steel Sterilizable Sampler (4)
Cooling Jacket
Agitator
Air Sparger

Other Features

(6) Collection of Contaminated Condensate and Sterilization before Release
(7) System Validation before Operation
(8) Monitoring Program
(9) Operation with Detailed Protocol

Submitted by the Eli Lilly Co.

Features of Standard Fermentor


Exhaust to Atmosphere Unfiltered
Single Seal
Dip Tube Harvest & Fill Line (3)
Compressed Air Inlet
Open Sampler (4)
Cooling Jacket
Agitator
Air Sparger

Submitted by the Eli Lilly Co.

Attachment C

THIRD MEETING OF THE INDUSTRIAL PRACTICES SUBCOMMITTEE OF THE FEDERAL INTERAGENCY ADVISORY COMMITTEE ON RECOMBINANT DNA RESEARCH

THURSDAY, SEPTEMBER 4, 1980

HAZARDS OF MAKING THE SAME PRODUCT BY RECOMBINANT DNA AS COMPARED TO OTHER METHODS.

GEORGE S. GORDON

A CHEMICAL PLANT IS A POTENTIALLY DANGEROUS PLACE TO WORK. BESIDES THE PHYSICAL EFFECTS OF BEING EXPOSED TO CORROSIVE, POISONOUS, OR OTHERWISE DANGEROUS CHEMICALS, THERE IS ALWAYS THE CHANCE OF EXPLOSION, PRESSURE VESSEL FAILURE, FIRE, ESCAPING STEAM, AND OTHER CATASTROPHIC ACCIDENTS. HOWEVER, BECAUSE OF AWARENESS OF THESE POTENTIAL HAZARDS, AND BECAUSE THE CHEMICAL INDUSTRY HAS LIVED WITH THEM FOR MANY YEARS, STANDARD DESIGNS AND OPERATING PROCEDURES HAVE LONG SINCE BEEN DEVELOPED FOR HANDLING TOXIC AND CAUSTIC CHEMICALS AND FOR AVOIDING CATASTROPHIC ACCIDENTS. (I INCLUDE FERMENTATION PROCESSES AS PART OF THE CHEMICAL INDUSTRY.)

THE RESULTING SAFETY RECORD IS A GOOD ONE, ACCORDING TO THE NATIONAL SAFETY COUNCIL. OUT OF 42 INDUSTRIES COVERED IN ITS MOST RECENT SURVEY, CHEMICALS RANKED FIFTH IN SAFETY, BEHIND RELATIVELY NONHAZARDOUS INDUSTRIES SUCH AS COMMUNICATIONS, ELECTRONICS, AIRCRAFT MANUFACTURE AND TEXTILES.

I HAVE ATTEMPTED TO COMPARE THE HAZARDS POSSIBLE IN THE OPERATION OF A FEW PETROCHEMICAL PROCESSES, USING ETHYLENE AS THE BASIC RAW MATERIAL, WITH POTENTIAL HAZARDS FROM FERMENTATION PROCESSES INVOLVING RECOMBINANT DNA, USING THE SAME RAW MATERIALS AND FORMING ESSENTIALLY THE SAME PRODUCTS. ALSO, I HAVE COMPARED PRECAUTIONS TAKEN DURING STANDARD FERMENTATION OPERATIONS WITH THOSE LIKELY TO BE TAKEN IN RUNNING LARGE-SCALE FERMENTATIONS IN WHICH RECOMBINANT DNA PROCEDURES ARE INVOLVED IN ONE WAY OR ANOTHER.

FERMENTATION PROCESSES ARE LESS SUBJECT TO CATASTROPHIC ACCIDENTS (EXPLOSION, FIRE) THAN ARE MANY PETROCHEMICAL OPERATIONS, BUT CAN, OF COURSE, BE EXTREMELY DANGEROUS WITH REGARD TO CHRONIC EXPOSURE. HERE AGAIN, THOUGH, SINCE FERMENTATION HAS BEEN USED ON A LARGE SCALE FOR MANY YEARS, PROCEDURES HAVE BEEN DEVELOPED WHICH HAVE KEPT ACCIDENTS TO A VERY LOW MINIMUM.

PETROCHEMICAL PROCESSES

TYPICAL OF SUCH OPERATIONS ARE THE PREPARATION OF ETHYL ALCOHOL FROM ETHYLENE, AND THE PREPARATION OF ETHYLENE OXIDE AND ETHYLENE GLYCOL FROM THE SAME STARTING MATERIAL:

ETHYL ALCOHOL

1. $C_2H_4 + H_2O \xrightarrow{\text{CATALYST}} C_2H_5OH$

CATALYSTS ARE PHOSPHORIC ACID, ALUMINUM OXIDE GEL, OR TUNGSTIC ACID ON SILICA GEL.

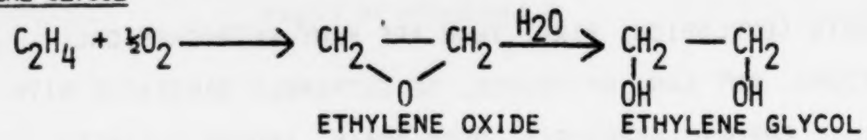
TEMPERATURES MAY REACH 400° C, AND THERE MAY BE PRESSURES UP TO 1000 PSIG.

AT SUCH EXTREME TEMPERATURES AND PRESSURES, FAILURE OF REACTION VESSELS OR LINES WOULD INDEED BE CATASTROPHIC WITH EXPLOSION AND FIRE PROBABLE.

2. $3C_2H_4 + 2H_2SO_4 \longrightarrow C_2H_5HSO_4 + (C_2H_5)_2SO_4$

THE ETHYL SULFATES ARE THEN HYDROLYZED TO ETHYL ALCOHOL AND SULFURIC ACID. HERE AGAIN, HIGH TEMPERATURES AND PRESSURES ARE INVOLVED, WITH CONSEQUENT DISASTROUS EFFECTS IN CASE OF REACTOR FAILURE.

ETHYLENE GLYCOL



IN THE FIRST STEP, A SILVER CATALYST IS USED, WITH TEMPERATURES REACHING 200° - 300° C, AND PRESSURES OF 300 PSIG. THE SECOND STEP IS ACID HYDROLYSIS, WITH RELATIVELY LOW TEMPERATURES (ABOUT 100° C).

AGAIN, TEMPERATURES AND PRESSURES ARE HIGH ENOUGH TO PRESENT A SERIOUS THREAT OF CATASTROPHE IN CASE OF REACTOR FAILURE.

FERMENTATION PROCESSES

HERE, THE SAME PRODUCTS COULD BE MADE FROM THE SAME BASIC STARTING MATERIAL, ETHYLENE.

ETHYL ALCOHOL

ONE APPROACH HERE IS TO DEVELOP A SUPERIOR STRAIN OF YEAST VIA THE RECOMBINANT DNA ROUTE, ONE WHICH WOULD PROVIDE YIELDS AND RATES OF REACTION COMPETITIVE WITH THE PETROCHEMICAL METHOD WHICH HAS MORE OR LESS SUPERCEDED THE ORIGINAL FERMENTATION APPROACH. THE FERMENTATION WOULD BE ACCOMPLISHED BY INOCULATION OF THE SUGAR SOLUTION WITH LIVE ORGANISMS, MUCH AS ALCOHOLIC LIQUORS HAVE BEEN MADE FOR THOUSANDS OF YEARS.

ETHYLENE OXIDE AND ETHYLENE GLYCOL

HERE, THE REACTION BETWEEN ETHYLENE AND OXYGEN WOULD BE BASICALLY THE SAME AS DESCRIBED ABOVE FOR THE HIGH-TEMPERATURE, HIGH-PRESSURE CATALYTIC PROCESS. THE CATALYST, HOWEVER, WOULD

CONSIST OF IMMOBILIZED ENZYMES, PREPARED ON A RELATIVELY SMALL SCALE USING GENETICALLY ENGINEERED ORGANISMS. THE ETHYLENE TO ETHYLENE OXIDE STEP (THE ONE USING THE IMMOBILIZED ENZYMES) WOULD BE CARRIED OUT AT LOW TEMPERATURES AND PRESSURES. THE SECOND STEP, FROM ETHYLENE OXIDE TO ETHYLENE GLYCOL, WOULD BE IDENTICAL TO THE ONE USED IN THE PETROCHEMICAL PROCESS.

PRECAUTIONS TAKEN

1. PETROCHEMICAL -

REACTIONS SUCH AS THESE, RUN AT HIGH TEMPERATURES AND PRESSURES, HAVE BEEN SAFELY HANDLED FOR MANY YEARS. THE USE OF HEAVY CONSTRUCTION MATERIALS, FREE OF CORROSION PROBLEMS, FREQUENT EQUIPMENT INSPECTIONS, REDUNDANT CONTROLS, AND EXCELLENT OPERATOR TRAINING, HAVE RESULTED IN A VERY LOW ACCIDENT RATE IN THE CHEMICAL INDUSTRY FOR THIS TYPE OF OPERATION.

2. FERMENTATION -

BECAUSE OF THE MODERATE PHYSICAL CONDITIONS INVOLVED IN THESE FERMENTATIONS, LITTLE CHANCE EXISTS FOR CATASTROPHIC ACCIDENTS, SO LONG AS THE FLAMMABLE MATERIALS SUCH AS ETHYLENE ARE NOT ALLOWED TO ESCAPE. STANDARD PROCEDURES HAVE BEEN DEVELOPED FOR ANY FERMENTATION OPERATION, AND FURTHER PRECAUTIONS ARE APPLIED TO FERMENTATIONS INVOLVING RECOMBINANT DNA, EVEN THOUGH THERE MAY BE NO ADDED DANGER. CONTAINMENT FOR RECOMBINANT DNA LARGE-SCALE PROCESSES INVOLVE AT LEAST THE FOLLOWING:

- For inoculation or sampling, sterilization of all lines with steam, before and after transfer.
- Use of double steam seals for agitators
- Use of an alarm system for any leakage to the atmosphere.
- Multiple filtration of any exhaust air (a) cyclone; (b) coalescent filter; (c) two sterile filters in series.
- Chemical or thermal kill at the finish of any operation;
- Implantation of batch sample in culture, after sterilization of the batch.
- Continuous monitoring of all exhaust systems, after filtration.
- Use of closed systems for inoculation or sampling.
- Incorporation of redundancy in operating protocols.
- Thorough indoctrination of operators, not only in procedures, but in recombinant DNA background.
- Continuous medical surveillance of all operators.

Any industrial process can be hazardous if carelessly carried out. However, use of such precautionary steps has reduced hazards, either of standard fermentation techniques or recombinant DNA fermentation processes, to a very low level.

Dangers of large-scale fermentations, using organisms or enzymes via recombinant DNA techniques, are similar to the dangers, if any, met at a laboratory scale. With proper physical and

BIOLOGICAL CONTAINMENT, FOLLOWING THE NIH GUIDELINES, THERE SHOULD BE LITTLE DANGER FROM THE PROCESSES DESCRIBED ABOVE. IN FACT, PRODUCTION-SCALE OPERATIONS MAY BE SAFER THAN THOSE IN THE LABORATORY, BECAUSE:

- o HEAVY STAINLESS VESSELS ARE USED, RATHER THAN GLASS.
- o COMPLETE CONTAINMENT IS USED FOR ANY TRANSFER OPERATIONS.
- o RAPID STERILIZATION IS POSSIBLE IN CASE OF A PROCEDURAL MISTAKE.

SUMMARY

CONTAINMENT AND OTHER SAFETY PROCEDURES HAVE LONG BEEN STANDARD FOR BOTH FERMENTATION AND PETROCHEMICAL PROCESSES, ON A PLANT SCALE. ACCIDENTS ARE EXTREMELY RARE IF ESTABLISHED PROTOCOLS ARE FOLLOWED. HOWEVER, IF THE REMOTELY POSSIBLE HAPPENS (E.G., FAILURE OF AUTOMATIC CONTROLS, RUPTURE OF METAL REACTION VESSELS, ETC.), CATASTROPHE CAN RESULT. BECAUSE LESS EXTREME CONDITIONS EXIST WHEN LARGE-SCALE FERMENTATION REACTIONS ARE CARRIED OUT THAN PREVAIL FOR MANY STANDARD PETROCHEMICAL PROCESSES PRODUCING THE SAME PRODUCTS, CATASTROPHIC ACCIDENTS ARE LESS LIKELY IN THE FORMER CASE. HOWEVER, IN LARGE-SCALE REACTIONS INVOLVING RECOMBINANT DNA PROCESSES, THE INDICATIONS ARE THAT EXTRAORDINARY PRECAUTIONS ARE BEING TAKEN TO AVOID THE ESCAPE OF ANY VIABLE ORGANISMS, AND TO COMPLY WITH THE NIH GUIDELINES.

Page 1 of Attachment D

EXECUTIVE OFFICE OF THE PRESIDENT
OFFICE OF MANAGEMENT AND BUDGET
WASHINGTON, D.C. 20503

September 23, 1980

MEMORANDUM FOR: DR. DONALD S. FREDERICKSON
DIRECTOR, NATIONAL INSTITUTES OF HEALTH

FROM: Dr. Gilbert S. Omenn GSO
Chairman, Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research

SUBJECT: Interim Report of the Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research

This memorandum constitutes an interim report from the Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research, to you, as Chairman of the parent committee.

Our subcommittee has now met three times -- April 1, June 18, and September 4, 1980. Appended are the minutes of these three meetings. As you can see from the minutes, we have had detailed presentations from the National Institutes of Health, Occupational Safety and Health Administration, National Institute of Occupational Safety and Health, Food and Drug Administration, Environmental Protection Agency, Department of Commerce, Department of Agriculture, and the Office of Technology Assessment. In addition, we heard presentations from invited representatives of the American Federation of Labor and Congress of Industrial Organizations, Oil, Chemical and Atomic Workers International Union, Pharmaceutical Manufacturers Association, and Eli Lilly and Company.

We are closely monitoring developments, such as:

- The results of NIOSH site visits to industry and its development of recommendations on worker education and medical surveillance;
- The deliberations of the NIH Recombinant DNA Advisory Committee on possible changes in its procedures for reviewing over-10-liter submissions;
- EPA risk assessment studies;
- FDA's preparations to review submissions involving products made with recombinant DNA techniques;
- OTA's study "Impacts of Applied Genetics."

I believe we are diligently carrying out our charge, as cited in your March 12, 1980, memorandum to me, i.e.,

> "In considering issues of occupational health and other federal responsibilities attendant upon industrial applications of recombinant DNA technology, the Subcommittee has the responsibilities:
>
> > To consider comments directed to member agencies concerning these issues;
> >
> > To provide a point of contact on these issues for persons outside the Federal Government and, where appropriate, to seek their advice, consultation, and participation in the work of the Subcommittee;
> >
> > To monitor and, where appropriate, to participate in the activities of the Recombinant DNA Advisory Committee and other activities of federal agencies pertaining to these matters."

I am impressed with the participation of the many federal agencies in our Subcommittee meetings. Much useful exchange of information and viewpoints has occurred. We have shared the reports and minutes of our meetings with all interested parties. I believe it is important for our Subcommittee to continue meeting to monitor the ongoing work of the member agencies and the non-Federal developments in this important area of Science and Technology. With the concurrence of the Subcommittee, I have scheduled another meeting to be held in December. I will then plan to continue to hold meetings approximately every three or four months until the parent committee judges that we have completed our mission.

Attachments
Minutes of Meetings

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20205

OCT 6 1980

MEMORANDUM FOR: Dr. Gilbert S. Omenn
Chairman, Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research

FROM: Dr. Donald S. Fredrickson
Chairman, Federal Interagency Advisory Committee on Recombinant DNA Research

SUBJECT: Interim Report of the Industrial Practices Subcommittee

Thank you for your September 23 memorandum constituting an interim report of the Industrial Practices Subcommittee of the Federal Interagency Advisory Committee on Recombinant DNA Research.

I, too, am impressed with the participation of the many Federal agencies in the Subcommittee meetings and the useful exchange of information and viewpoints which has occurred. I agree that it is important for the Subcommittee to continue meeting to monitor the ongoing work of the member agencies, and the nonfederal developments in this important area of science and technology.

I appreciate your taking the time from your busy schedule to serve as Subcommittee Chairman.

INDUSTRIAL PRACTICES SUBCOMMITTEE
OF THE FEDERAL INTERAGENCY
ADVISORY COMMITTEE ON RECOMBINANT
DNA RESEARCH

Minutes of the Fourth Meeting*
Tuesday, December 16, 1980
1:00 to 3:00 p.m.
Room 3104, New Executive Office Building
Washington, D.C.

Chairman's Introduction

Dr. Omenn said that developments since the last subcommittee meeting, including the frenzied response on Wall Street on the first day of the Genentech public stock offering and the heavy press coverage of the Harvard University decision not to become a minority partner in a commercial venture to exploit recombinant DNA technology, show once again that this subcomittee is dealing with issues of great significance for science, industry, and society.

Report from OSHA

Dr. Adamson distributed copies of Dr. Bingham's September 24 letter to Dr. Fredrickson (Attachment B). As noted in the letter, Dr. Bingham estimates "that for completion of the NIOSH study and for implementation of OSHA's subsequent decisions, a period of approximately two years will be required."

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* A list of attendees is appended as Attachment A.

Report from NIH

Dr. Talbot distributed copies of the proposed First Annual Update of the NIH Program to Assess the Risks of Recombinant DNA Research (Federal Register September 17, 1980, pages 61874-61878). Following the public comment period it will be finalized and published again in the Federal Register.

He noted that the NIH Recombinant DNA Advisory Committee (RAC) met most recently on September 25-26, 1980. He distributed copies of the NIH Director's "Decision Document" accepting most of the recommendations made by the RAC at the meeting, accompanied by publication of a "clean copy" of the NIH Guidelines for Research Involving Recombinant DNA Molecules (Federal Register November 21, 1980, pages 77372-77409). The two major changes in the Guidelines, effective November 21, are:

1. For experiments under 10 liters, for which the containment conditions are given in the Guidelines, the previous requirement has now been eliminated that a "Memorandum of Understanding and Agreement" describing the experiment be sent to NIH by the Institutional Biosafety Committee.

2. For proposed experiments over 10 liters (these have been primarily voluntary submissions from industry), the RAC will continue to do pre-review of the biological aspects of the individual proposal, but will no longer do pre-review of the details of physical containment. A Working Group of the RAC, including representation from all interested federal agencies, will do post-review of the system and advise the RAC on possible future changes in the definition of the "P-LS" physical containment criteria.

On November 24-25, NIH sponsored a meeting of Chairpersons of Institutional Biosafety Committees. A number of suggestions from that meeting will be considered by the RAC at its next meeting, January 8-9, 1981.

Report from FDA

Dr. Miller reported on a number of steps which FDA is considering relative to its review and approval for marketing of products of recombinant DNA technology. Since there is different statutory authority for different product classes (i.e., drugs, foods, medical devices, biologics, veterinary medicines), it is likely that regulation will be on a product-by-product basis through the appropriate FDA Bureau. Substances produced by recombinant DNA technology will generally be treated as analogous products produced by conventional technology, with the qualification that quality assurance techniques may have to be tailored to assure continuous control of purity and identity of products. In addition, at least for the Bureau of Drugs and the Bureau of Biologics, for the present, a new Notice of Claimed Investigational Exemption for a New Drug (IND) and a new New Drug Application (NDA) will be required for a product made by recombinant DNA technology, even if identity is demonstrated with the natural substance (or with a previously approved drug, purified in a conventional way).

Dr. Omenn asked why a new IND and NDA would be required. Dr. Miller pointed out that the statutory definition of a "new drug" (21 U. S. Code 321(p)) is "any drug . . . the composition of which is such that such drug is not generally recognized, among experts qualified by scientific training and experience . . . as safe and effective . . . ." Until drugs made by recombinant DNA techniques become "recognized [by] . . . experts . . . as safe and effective," they will be treated as "new drugs." Dr. Miller said that industry agrees, at least for the first drugs made by recombinant DNA techniques, that the intense scrutiny provided by the IND process is in everyone's best interest. Some subcommittee members expressed concern that this FDA tentative decision could tend to discourage small companies with little IND experience, and could lead to unnecessary delays in getting these drugs to the public. Dr. Miller pointed out that, in fact, some companies are entering the IND process for the first time, and that evaluation by FDA of these submissions is on the "fast track."

Dr. Miller reported that FDA has issued a proposed rule (Federal Register, December 11, 1979, pages 71742-71749), but not as yet a final rule, concerning agency policies and procedures for complying with the National Environmental Policy Act (NEPA), including the preparation of environmental assessments and environmental impact statements. One approach being considered is to make it known to industry that FDA will view a manufacturer's compliance with the NIH Guidelines as mitigation of any potential adverse effects to the environment from this technology. Consequently, in most instances, when such assurances were made, FDA would not seek further environmental review in the form of an Environmental Impact Statement. Some subcommittee members said this was a desirable way to encourage compliance with the Guidelines; others, however, believed it might be interpreted as an inappropriate change in the current voluntary, good-faith compliance by industry.

Dr. Miller reported that the FDA conducts premarket inspections of manufacturing plants. On those inspections, if FDA recognizes a potential problem relating to the jurisdiction of another agency belonging to the Interagency Regulatory

Liaison Group, FDA has agreed under recently published guidelines (Federal Register, March 28, 1980, pages 20638-20641) to make such information available to the other agency. However, The Food, Drug and Cosmetic Act prohibits disclosure of proprietary information outside of the Department of Health and Human Services.

Report from NIOSH

At the previous Subcommittee meeting, Dr. Robbins distributed the report of a NIOSH walk-through survey conducted at Eli Lilly and Company Research Laboratories. He now distributed the reports of NIOSH walk-through surveys conducted at Genentech, Inc., South San Francisco, California, on April 8, 1980 (Attachment C), and at Cetus Corporation, Berkeley, California, on April 9, 1980 (Attachment D). A fourth survey at Burns-Biotec Laboratories, Inc., Elkhorn, Nebraska, on November 24, 1980, has been completed; the report is not yet available, although Mr. Elliott and Mr. Pauker described to the Subcommittee some of the findings. They said the walk-through survey was much more informative when the NIOSH team: (1) had received, prior to the visit, copies of the proposal to exceed 10 liters in volume, which the company had submitted to the NIH Recombinant DNA Advisory Committee; and (2) observed, during the visit, an actual production run. NIOSH may do follow-up surveys at Eli Lilly and Genentech to observe a production run and product purification steps. Mr. Pauker noted that there were differences in approaches at different companies in such areas as the physical set-up of the production unit, operational protocols, use of control technology, handling of exhaust air, employee health system, policy for workers receiving antibiotic therapy, and emergency plans.

Dr. Robbins reported that planning is moving ahead for a joint NIOSH-EPA project to assess the adequacy of currently used "control technology." A CDC-NIOSH recombinant DNA surveillance group met for the first time on October 23, and will make recommendations concerning: (1) medical surveillance of potentially exposed workers; (2) central collection and analyses of medical surveillance data for epidemiology; and (3) establishing an emergency response team.

Chairman's Summation

Dr. Omenn said that industry and government have behaved responsibly and have made hasty legislation unnecessary. He expects to leave government shortly. If so, the Director, NIH, will appoint a new Subcommittee chairman. The Subcommittee executive secretary will contact members concerning the next meeting. Dr. Omenn extended his best wishes to the Subcommittee members, and thanked them for their constructive participation in these fruitful meetings.

Bernard TALBOT

Bernard Talbot, M.D., Ph.D.
Subcommittee Executive Secretary
January 12, 1981

LIST OF ATTENDEES
Meeting of December 16, 1980

CHAIRMAN

Dr. Gilbert S. Omenn
Associate Director for Human Resources, Veterans, and Labor
Office of Management and Budget

EXECUTIVE SECRETARY

Dr. Bernard Talbot
Special Assistant to the Director, NIH

DEPARTMENT OF AGRICULTURE

Dr. Ralph McCracken
Associate Director, Science and Education

Dr. Gerald Still
Science and Education Administration

Dr. Sue Tolin
Science and Education Administration

DEPARTMENT OF COMMERCE

Dr. George Gordon
Office of Environmental Affairs

DEPARTMENT OF HEALTH AND HUMAN SERVICES

OFFICE OF THE ASSISTANT SECRETARY FOR HEALTH

Dr. Lowell T. Harmison
Science Advisor, Office of Environmental Affairs

Mr. Philip Janus
Staff Assistant, Office of Environmental Affairs

Mrs. Florence Hassell
Office of Health Planning and Evaluation

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH, CDC

Dr. Anthony Robbins
Director, NIOSH

Mr. Seth Pauker
Health Scientist, Office of Program Planning and Evaluation

Mr. Larry Elliott
Industrial Hygienist
Division of Surveillance, Hazard Evaluation, and Field Studies

NATIONAL INSTITUTES OF HEALTH

Dr. Robert McKinney
Chief, Occupational Safety and Health Branch
Division of Safety

FOOD & DRUG ADMINISTRATION

Dr. John Petricciani
Deputy Director, Division of Pathology
Bureau of Biologics

Dr. Henry Miller
Bureau of Drugs

Dr. Timothy J. Henry
Staff Science Advisor

DEPARTMENT OF LABOR

Dr. Lucile Adamson
Division of Health Standards, OSHA

Dr. Chia T. Chen
Directorate of Technical Support, OSHA

Mr. Peter Wall
Directorate of Technical Support, OSHA

ENVIRONMENTAL PROTECTION AGENCY

Dr. Darryl Banks
Executive Assistant to the Assistant
Administrator for Research and Development

Dr. Morris Levin
Director, Innovative Research Program

NATIONAL SCIENCE FOUNDATION

Dr. Herman Lewis
Head, Section on Cellular Biology
Division of Physiology, Cellular and Molecular Biology

EXECUTIVE OFFICE OF THE PRESIDENT

COUNCIL ON ENVIRONMENTAL QUALITY

Dr. Robert Harris
Member, Council on Environmental Quality

Page 1 of Attachment B

U.S. Department of Labor

Assistant Secretary for
Occupational Safety and Health
Washington, D.C. 20210

Dr. Donald Fredrickson
Director
National Institutes of Health
Bethesda, Md. 20205

SEP 24 1980

Dear Dr. Fredrickson:

As you know, both the NIH Recombinant DNA Advisory Committee (RAC) and OSHA, among others, have been concerned with how the recombinant DNA industry should be monitored in an attempt to protect workers from any existing or potential hazards which may be peculiar to this type of activity. OSHA has been actively reviewing the ways in which we can fulfill our mandate in this regard.

In recognition of our need to develop a regulatory policy concerning recombinant DNA, representatives of OSHA and NIOSH have been attending the RAC meetings and participating in the meetings of the Interagency Advisory Committee on Recombinant DNA Research and of its Industrial Practices Subcommittee. In addition, NIOSH has undertaken a study of the industry and its potential hazards, as decribed in the attached memorandum, which will provide a basis upon which OSHA regulatory decisions in this area can be developed. We estimate that for completion of the NIOSH study and for implementation of OSHA's subsequent decisions, a period of approximately two years will be required.

I regret that the RAC wishes to terminate its reviews of engineering plans for proposed private-sector DNA operations. Because this would remove one of the existing procedures for promotion of biological safety in these operations, I would like to describe to you how OSHA proposes to deal with recombinant DNA workplaces during the interim period, until OSHA regulatory policy for this industry is developed.

Until formal regulatory decisions are made, OSHA will regulate recombinant DNA operations under its current authority, standards, and guidelines. Thus, OSHA will respond to complaints from workers, unions, or others about recombinant DNA hazards by reviewing the complaints and conducting inspections as warranted. In addition, inspections can also be initiated by OSHA without receipt of a specific complaint if the workplace selection is made in conformity with OSHA's current guidelines on general schedule inspections. If an inspection of a recombinant DNA facility is undertaken, OSHA compliance officers will, consistent with OSHA established practice,

look for violations of existing standards and of the general duty provision (Section 5(a)(1)) of the OSH Act which states, in part, that the employer shall furnish a place of employment free from recognized hazards that are likely to cause serious physical harm. Furthermore, during such an inspection, OSHA may choose to determine whether the employer is in compliance with the RAC recommendations and with applicable sections of the NIH guidelines. OSHA will inform the employer of any observed failures of such compliance and will determine what OSHA action should be taken to bring about worker protection from recombinant DNA hazards at that workplace.

We hope that these procedures by OSHA, coupled with the continued function of the RAC and continued voluntary compliance by industry with RAC recommendations, will provide for safety of the workers in this developing industry.

Sincerely,

Eula Bingham

Eula Bingham
Assistant Secretary
Occupational Safety and Health

Page 3 of Attachment B

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

TO : Dr. Gilbert S. Omenn
Chairman, Industrial Practices Sub-Committee
of the Federal Interagency Advisory Committee
on Recombinant DNA Research

DATE: JUN 11 1980

FROM : Anthony Robbins, M.D.
Director, NIOSH

SUBJECT: NIOSH's Proposed Program for Addressing Potential Hazards to Workers Related to Commercial Recombinant DNA Applications

This memorandum outlines NIOSH's program for addressing potential hazards to workers related to commercial recombinant DNA applications. It is important to consider how NIOSH concerns fit into the concerns of other representatives on this committee and of the Recombinant DNA Advisory Committee for potential health hazards.

NIOSH is focusing its evaluations of potential hazards in the recombinant DNA area of the biotechnology industry for a variety of reasons. First, the number of workers involved in recombinant DNA applications is potentially higher than any other segment of the biotechnology industry (e.g., in vaccine or antibiotic production). Industries outside the pharmaceutical sector are also applying recombinant DNA techniques. These industries which are involved in such areas as energy development and chemical production have far less experience in handling biological agents and may be less aware of potential or actual biohazards.

Also, recombinant DNA applications are in the preliminary stages of capitalization. Recommendations for safeguarding health can be most cost-effective if installed now, avoiding expensive retrofit. Rarely do we have such opportunities for active prevention in the early stages of development of potentially hazardous processes.

NIOSH is initiating a program to help define the potential for health hazards to workers participating in commercial recombinant DNA applications. We will continue to advise OSHA of our findings and recommendations.

As part of this program we will continue the on-site surveys of companies moving into scale-up or pilot production phases of operation, in order to assess the control measures and reduce human exposures. We are including evaluation of the following areas as appropriate:

1) physical containment design (e.g., fermenters)
2) engineering controls (e.g., ventilation, filter of exhaust gases)
3) work practices (e.g., operational protocols, clothing and hygiene requirements)
4) validation procedures (e.g., sterilization and fermenter containment)

5) emergency procedures (e.g., spills)
6) environmental monitoring
7) medical surveillance (e.g., serum samples for serologic changes; monitor possible physiologic changes related to product exposure)
8) worker education

It will be necessary to request directly from the companies proposing work, those submissions which have been made under the NIH voluntary compliance program (See Attachment). These submissions describe the design of the physical containment facilities, work practices and biological systems which are intended to ensure safe operation of the production process. As NIH can attest, adequate safety evaluation is impossible without this information.

In order to support our evaluation, we will seek information about fermentation processes which use similar equipment to that proposed for recombinant DNA fermentation application. Data obtained on fermenter reliability and effectiveness, accidents and possible routes of exposures should help us to determine whether potential health hazards in other biotechnology applications need assessment.

Appropriate medical surveillance has been discussed by different groups. The Recombinant DNA Advisory Committee has discussed the need for medical surveillance and NIOSH has been asked for recommendations by some of the companies we have visited. The varied nature of proposed host-vector systems and the multitude of possible biologically active products and byproducts make the determination of appropriate and effective medical surveillance a complex issue. To help address this need, NIOSH, together with other CDC experts in infectious disease, epidemiology and molecular biology will expeditiously develop guidelines for medical surveillance techniques.

It would be helpful if those companies proceeding with "large-scale" recombinant DNA applications inform NIOSH, so that the Federal agencies charged with the protection of public health will have basic information upon which to act in the event hazards unexpectedly emerge. This notification to NIOSH should at a minimum contain the name and location of the company, the host-vector system utilized, and the product which will be produced. The companies should also maintain internally complete records of their projects. The records should detail such information as the biological system, physical containment level, design and configuration, and identification of workers with their time associated with the projects. It is important to bear in mind that the retention of this type of information by the companies should not be unduly burdensome, because they would invariably be needed for the project development. This information would be necessary to evaluate any hazards which might unexpectedly emerge.

After the completion of the preliminary site-visit program described above, we are also proposing to study, as indicated by industry information, any particular projects which might have a higher potential for hazard or for guarding against it.

We estimate that NIOSH will publish general recommendations next year. Our recommendations will be based upon the assessments conducted and the current level of knowledge in this area. This publication will detail work practices and control technology which will minimize exposures to workers from potential hazards.

Attachments
cc: Members of Committee

Walk-Through Survey Report
of
Genentech, Inc.
South San Francisco, California

DATE OF SURVEY:
April 8, 1980

REPORT WRITTEN BY:
Larry Elliott

SURVEY TEAM:
George Carson
Larry Elliott
Seth Pauker
Dave West

DATE OF REPORT:
October 16, 1980

Industrial Hygiene Section
Industry-wide Studies Branch
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health
Cincinnati, Ohio

PLACE VISITED:	Genentech, Inc. South San Francisco, California
DATE OF VISIT:	April 8, 1980
PERSONS CONDUCTING SURVEY:	George Carson Larry Elliott Seth Pauker David West
EMPLOYER REPRESENTATIVE CONTACTED	Robert Swanson, President (415) 952-0123
UNION:	None
STANDARD INDUSTRIAL CLASSIFICATION OF PLANT:	2834 Pharmaceutical Preparations
PURPOSE OF SURVEY:	To investigate and evaluate Genentech's methods of containment, environmental monitoring, and health surveillance for production/research processes utilizing recombinant DNA techniques.

ABSTRACT

A walk-through industrial hygiene survey was conducted at the Genentech Research Laboratories in South San Francisco, California on April 8, 1980. This company is involved in research and development of recombinant DNA fermentation processes applicable to pharmaceutical preparations. Assessments of control technology, including containment design, validation procedures, and work practices were made during the visit. Recommendations were made to strengthen specific areas in order to minimize potential hazards to the worker.

INTRODUCTION

The public debate over genetic engineering has focused on the possible hazards of genetically modified microorganisms, potential health hazards to workers involved with industrial application of recombinant DNA techniques, and the utilitarian prospects of such technology. Several risk assessment programs designed to investigate some of the characteristics of proposed host-vector systems which might effect hazard potential will be conducted by interested scientists. Likewise, the benefits from recombinant DNA technology are being as vigorously promoted. The National Institute for Occupational Safety and Health (NIOSH) is responsible for conducting research, recommending occupational safety and health standards, providing technical assistance to workers and employers, and conducting training and educational programs. NIOSH's responsibility extends to both existing and emerging technologies which might impact on worker health and safety. Thus, NIOSH is evaluating the potential occupational hazards involved with recombinant DNA technology.

This research effort was prompted by the anticipated surge of recombinant DNA techniques in various industrial processes. Genetic engineering technology may be utilized in various manufacturing processes in the areas of agriculture, organic chemicals, energy, food processing, and pharmaceuticals. This potential growth and the possibility of uncharacterized occupational exposures indicate the necessity for careful evaluations of health risks. NIOSH is accustomed to examining new technologies for potential occupational hazards and developing recommendations for safeguarding the workers health. Implementation of safeguards and protective engineering controls early in the growth of an industry can only minimize human suffering and avoid expensive retrofitting of production systems.

Under the Occupational Safety and Health Act of 1970, Public Law 91-596, NIOSH was mandated and authorized to conduct research and health studies. Specifically, section 20(a)7 states that NIOSH shall conduct and publish industry-wide studies of the effects of chronic or low level exposure to industrial materials, processes, and stresses on the potential for illness, disease, or loss of functional capacity. Thus this research is pursuant to the development of health standards applicable to a broad range of occupational environments. In compliance with this mandate, the Industry-Wide Studies Branch of the Division of Surveillance, Hazard Evaluations and Field Studies is conducting a research study to assess the potential occupational hazards in research and the commercial application of recombinant DNA technology.

In order to analyze the current technology for potential occupational hazards, NIOSH plans to conduct several walk-through surveys of recombinant DNA processes and associated industries. Identification of potential hazards, with recommendations to minimize worker exposure, will be derived from the information gained during these surveys. This report contains recommendations relevant to operations at Genentech, Inc. Recommendations applicable to the entire industry employing recombinant DNA technology will be made after NIOSH completes a thorough assessment. An assessment of the following areas will be made:

- process operations with attendant potentials for worker exposure
- engineering controls (e.g., physical containment design, ventilation, exhaust gas filtration, waste product controls, etc.)
- validation procedures (e.g., of sterilization, physical containment, and process termination)
- work practices
- emergency and accident procedures
- medical surveillance
- environmental monitoring
- number of exposed workers
- employee training and education

This survey of Genentech, Inc. in South San Francisco was conducted as one of a series of initial walk-through surveys of firms utilizing recombinant DNA techniques in research or production.

DESCRIPTION OF THE COMPANY

Genentech started in 1976 as an independently owned research organization. Research efforts by Genentech, utilizing recombinant DNA, have resulted in somatostatin production (1977), human insulin (1978), and human growth hormone (1979). Genentech is now in a transitional phase moving from strictly research and development toward manufacturing and production. Genetic engineering research and development of production applications is the prime objective of this company. Research projects are generated from within the company organization or collaborations with other companies for product or process development.

Genentech started operations in the South San Francisco facility in 1978 and has experienced rapid expansion. On-going construction in the facility is trying to meet the demands of this growth.

Genentech's organizational structure consists of four scientific divisions. The Nucleic Acid division is involved in gene synthesis and organic chemistry research. The division of Molecular Biology conducts research in cellular cloning, preparation of complementary DNA, messenger RNA, expression of plasmids, and the study of genetic structure. A third division, Protein Research and Process Development, is concerned with fermentation research and development, protein biochemistry research, and total process development. The fourth scientific division, Biology, conducts animal studies and designs appropriate animal models for testing.

DESCRIPTION OF THE WORKFORCE

Currently Genentech employs approximately 80 persons of which about 68 are involved directly with research and process development. Very few personnel are actually designated as operators of fermentation systems. Manpower requirements for monitoring fermentation processes and systems are anticipated to be no more than 10-15% of the total workforce. There is no structured work schedule requirements for most employees; most work is conducted during regular daytime hours. Two employees currently work a night shift.

DESCRIPTION OF THE PROCESS

Process development for the production of A & B insulin chains, proinsulin, and somatostatin is now underway. Development of fermentation and extraction techniques are also being conducted. Production processes utilizing recombinant DNA will vary depending on the nature of the product desired and the steps required to achieve the product. General process descriptions for fusion proteins and direct expression techniques are outlined in Diagram 1 and 2 respectively.

Genentech's fermentation capacity consists of shake flask, 10 liter, and 600-liter fermentation systems. The 10 liter fermentors are glass and are located in the main production room with the 600-liter fermentor. The 10-liter fermentors are inoculated using a shake flask. Inoculation of the 600-liter fermentor may be accomplished via shake flask or from the 10-liter fermentor by transporting the inoculum in a glass flask with an attached needle to introduce the contents of the flask into the fermentor through a diaphram port. The 10-liter and 600-liter fermentors are loaded with nutrients through continuous piping from feed tanks. The feed tanks and lines are a closed system that is sterilizable and maintained under positive pressure (about 10 p.s.i.). One feed tank is filled with a chemical killing agent for inactivation of the process for emergency measures.

The fermentation process is monitored and terminated when optimum cell growth is attained. Termination may be by chemical or physical means depending on the product or process. Harvest of the cells is accomplished by centrifugation followed by physical lysis of the cells. The crude product undergoes purification and extraction processes such as chromatography. The extraction, cell lysing, and product purification processes were not observed during this survey. These processes should be observed and evaluated for potential hazards when the company has developed them to the state of continuous production.

DESCRIPTION OF INDUSTRIAL HYGIENE, SAFETY AND MEDICAL PROGRAMS

Industrial Hygiene

This company has no industrial hygienist on staff. Environmental monitoring has been limited to open agar plate sampling for settling aerosol particles and air sampling by impaction on agar plate techniques. The air impaction technique was used for containment certification of the fermentation system for submission to NIH and is not a routine measurement. Results of the open agar plates have been recorded on the fermentation batch records since October 1979. The open agar plates are placed next to the agitator seal of the 600-liter fermentor during each batch. This seal would appear to be the most likely source of leakage, should it occur during the fermentation run. Potential for leakage, or aerosol production, may also exist due to the methods and equipment used for inoculation and sampling of the fermentors. No viable organisms have been recovered by this method at the time of the survey.

Safety and Health

A formal safety program is being development by the Director of Human Resources. The company has a safety committee and specific guidelines for work practices, housekeeping, safe laboratory practices, and emergency procedures are being drafted.

An Institutional Bio-Safety Committee (IBC) has been formed in compliance with the NIH guidelines. The Bio-Safety officer is a member of the company safety committee. A local physician and a consulting environmental engineer are also members of the IBC.

Employees are supplied laboratory coats as protective clothing. These coats are laundered outside the company by a contract laundry. Employees are required to wear the coats in accordance with the NIH guidelines. Showers and change rooms are available for emergency use.

Medical Program

The company is now developing a formal medical program for employee health surveillance. This program is being developed under the direction of the Director of Human Resources and a consulting physician who is a specialist in industrial medicine. They are arranging emergency clinical support, preemployment physicals, and serum sample aquisition and storage. Employees on antibiotic therapy are required to report to their supervisor and are excluded from working with recombinant DNA organisms. Production workers involved in the production of human growth hormone and interferon will be subject to fecal monitoring.

This company has recorded no lost time accidents in its four year history.

DESCRIPTION OF PAST EXPOSURES AND POTENTIAL HEALTH HAZARDS

Data on exposure of employees to recombinant DNA, organisms utilized in fermentation processes is limited to the environmental monitoring previously mentioned. No quantitative results are available to document range or incidence of exposure.

Other potential health hazards are radioisotopes and organic chemicals such as chloroform, pyridine, formic acid, and formaldehyde. Proper usage, storage, and disposal requirements have been discussed by the company with the employees involved with these hazards.

EVALUATION OF THE PLANT AND PROGRAMS

Physical Containment Design

Physical containment laboratories at the P-1 and P-3 levels, used for research and development of recombinant DNA techniques were toured. The design and operation of these laboratories were intended to be within the NIH guidelines. Genentech has experienced rapid growth and expansion with apparent priority given to advancement of the scientific and production programs. The result is a poor location of production areas and poor arrangement of equipment within the production area. On-going construction and facility modifications will possibly continue as the production process develops. A biosafety consultant is utilized by the company whenever changes are made in recombinant DNA containment facilities.

Areas of the production room were under construction at the time of this survey. This production room is designed to meet the NIH requirements for P1-LS level of containment with additional structural features that could qualify it as a P-3 laboratory. This room houses the 600-liter (metal) and 10-liter (glass) fermentors. Single pass ventilation, negative air pressure in the room, floors sloped to drains in order to contain large spills, and controlled entry are additional features not required by NIH for P1-LS. Non-porous and readily cleanable walls, floor, and ceiling are also planned. However, several problems exist in the production area that may possibly be corrected when the program requirements are achieved.

The production room is immediately adjacent to high traffic areas and the public reception area of the facility. This problem is compounded by a "fire door" (opens into the room) that accesses into the hallway from the public reception area. The door was locked and only openable from inside the production room. Sloped floors have been installed in the room, yet none of the access doors had thresholds. Feasibly a large spill could flow under the door.

The integrity of the containment design may be compromised by the 10-liter fermentors being glass, the shake flask method of innoculating the large fermentor, and the agitator drive and seals of the 600-liter fermentor being bottom-mounted.

Engineering Controls

The ventilation systems, fermentor exhaust gas filtration, waste products control, biological safety cabinets, access barriers, and all interior room surfaces in the P-3 and P-1 laboratories with the NIH guidelines.

The NIH guidelines for the P1-LS containment level do not require specific ventilation measures or distinct room confinement. Genentech has added these features to their production area. Exhaust gases pass through a ceramic filter and then an incinerator. Air entering the system also passes through a ceramic filter.

Sterilized liquid wastes from the fermentation system are drained to the public sewer. None of the process materials are recycled or treated (other than sterilization by live steam or addition of the chemical killing agent) prior to disposal. Solvent and radiation wastes are handled separately and are not disposed of in the public sewer.

Validation Procedures

The sterilization process is validated for thorough activity anually. Steam sterilization effectiveness is checked during each batch with indicator crayons or strips. These indicator methods only depict, at best, if the required sterilization temperature was reached at a given location. They do not identify areas of the system that may not have been thoroughly sterilized.

The nutrient lines and feed tanks are sterilizable, but validation of sterilization effectiveness in this system is not conducted.

Exhaust gas systems are visually checked when cleaned. Millipore filter air sampling techniques will be used to evaluate performance of these systems.

All biological safety cabinets receive scheduled performance testing and certification. Autoclaves are monitored by recording charts to document performance.

Centrifuge equipment and the Martin Gaulin (French) press receive periodic maintenance checks.

Work Practices

Specific guidelines for safe work practices are being drafted by the safety committee. Good laboratory technique and safe work practices are now left to the individuals' experience and the direction of immediate supervisor.

Operators of the fermentation systems are trained in the use of the equipment, the process methods, and safety precautions. Operators are required to use check lists for starting, running, and finishing each batch. Emergency procedures are outlined on these check lists.

Evidence of eating and drinking in laboratory areas was noticed during the facility tour. Laboratory coats and gloves were not consistantly used by the employees as several employees were observed working without laboratory coats. The dual function design of the production area (P-3 or P1-LS) could conceivably cause work practice problems. If this area is designed to function as a P-3 level containment laboratory then actual practices and procedures for controlled access should be followed. Unrestricted traffic flow to the 600-liter fermentor should be discouraged unless distinct procedures are devised to maintain the integrity of use as a P-3 facility or a production P1-LS area.

The company does not have a set safety policy or procedure outlined for maintenance performed in laboratories or on fermentation systems.

Cleaning of the laboratories and the production area is conducted by the employees in these areas. Formaldehyde is used to sanitize the surfaces in the production room. Paraformaldehyde is vaporized on a hot plate to fill the room with formaldehyde vapor. Personnel performing this operation use a full face protective mask with a replaceable organic vapor cartridge.

Emergency and Accident Procedures

Plans for emergencies, laboratory accidents, and safety precautions are being drafted by the company safety committee.

CONCLUSIONS

General experience has demonstrated that safe conduct of research or production processes involving hazardous entities is dependent on good work practices, availability and use of effective control technology, and effective management. Microbiological Research requires containment features sufficient enough not only to exclude entry of contaminants into, but also prohibit escape of organisms from the system. Thus, attention to exposure control must receive equal emphasis as process integrity and production development. Safety and health, medical surveillance, and emergency programs need to be implemented and managed with the same degree of attention that the scientific production programs receive.

RECOMMENDATIONS

Genentech needs to implement safety and health, medical surveillance and emergency programs and procedures immediately. These should be documented, understood by all concerned, and specific for Genentech's operations. These programs should receive the same managerial attention and administrative support as the production and scientific programs. Emergency guidelines, laboratory practices, personal hygiene habits and practices, protective clothing and equipment requirements, maintenance procedures, and medical surveillance are some specific areas where documented guidelines are needed. Such guidelines will serve only as well as they are responsibly implemented. Without effective management and supervisory control these guidelines will only be ideals and not practices.

> A medical program should consist of preemployment and annual physical examinations in order that employees may be monitored for abnormal health effects. Immunizations should be considered for employees working with known pathogens. Employees reporting to work from sick leave (after 3 or more days) should have the medical clearance of a physician. Employee records should be maintained and include notes on all illnesses, operations, physical disorders, theraputic medications, and accidental exposures.
>
> A systematic scheduled preventive maintenance program for agitator seals, control valves, pressure relief valves and equipment/facility safeguards should be implemented. Sterilization of fermentor, feed lines, feed tanks, inoculating device, exhaust p[illegible]e, sampling ports, and extraction devices must be validated by a procedure that demonstrates effective sterilization.

A registry of all workers employed with recombinant DNA process should be established and maintained for reference to determine any abnormal medical problems or unusual occurances that someday may be attributed to recombinant DNA processes. NIOSH has had considerable experience in designing and maintaining such worker registries and can thus provide assistance in design, implementation, and logistical requirements. The comparability of individual company registries is desirable for optimum and successful surveillance on an industry wide basis.

Biological monitoring of worker body fluids for specific organisms carrying recombinant DNA molecules is costly and technically demanding. Quantitative and qualitative sampling of air and surfaces in the production and laboratory areas can be an effective measure of sanitation, environmental conditions, and efficacy of control measures. Environmental sampling programs have been utilized in hospitals, the food industry, and containment laboratories for years and the principles of these programs are applicable to the operations at Genentech. The efficacy, sensitivity, and accuracy of environmental sampling and analytical techniques should, however, be considered and their validity must be established prior to utilization. The procedures analysis, sampling points and sampling schedules, thus established, should be formalized in writing.

Containment controls could be enhanced by designing an unbreakable inoculating device for large fermentors. It should be capable of being sterilized after inoculating but prior to detachment from the fermentor. The presently used shake flask method of inoculating large fermentors is unwieldy and provides potential for exposure by accidental breakage, spillage, or aerosol production during detachment from the fermentor. Replacement of the glass 10-liter fermentors with metal cased ones would also preclude accidental exposure due to breakage of the glass. Repositioning of the agitator drive and seals from the bottom of the fermentor to the top should be evaluated. This may facilitate better engineering design and ease of maintenance. A leaking seal, in the bottom of the fermentor, could allow drainage of culture liquid resulting in a substantial exposure.

Physical containment of the fermentation process, up to and including inactivation of the culture is mandatory. Physical containment of the biological material within a closed system should, preferrably, also extend through the cell lysis and product extraction phases. Capital investment for this now would not only enhance production aspects but also would minimize potential for worker exposure to products.

The P1-LS production area should be segregated from other production or research operations. This would maintain the integrity of the process and procedures outlined for recombinant DNA fermentation processes. Such system isolation will also enhance the containment of the entire operation to preclude accidental exposure to, and from, other employees and processes.

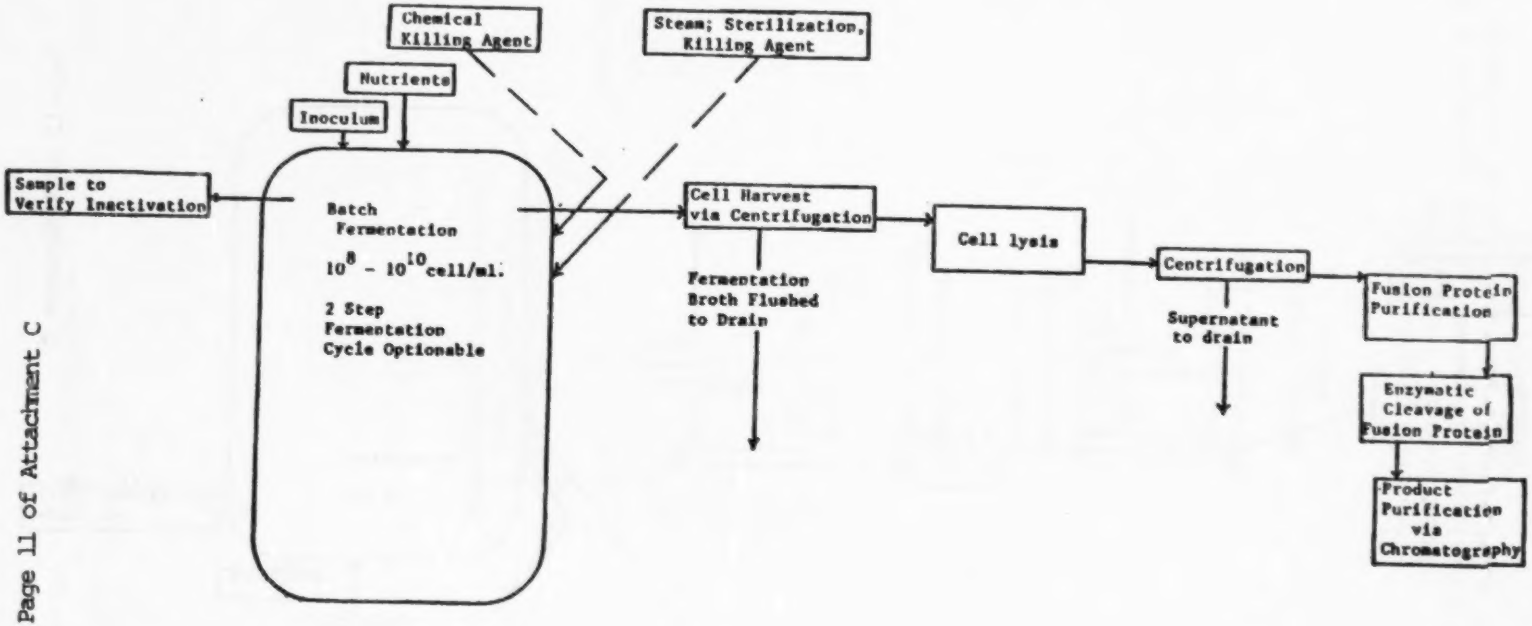
Specific procedures whould be employed for handling chemical and radiological wastes. Only personnel trained in these procedures should handle the wastes. Protective clothing should be decontaminated, or marked hazardous so the laundry may handle these garments appropriately.

The use of formaldehyde as a disinfecting agent in laboratory areas merits certain precautions. Employees should wear rubber gloves when working with formaldehyde and when contact with the hands is likely. If the disinfection process involves occasional brief exposures to formaldehyde at concentrations in excess of 1.2 mg/cubic meter an appropriate respirator should be used. A respirator training program would then also be required, with emphasis on cleaning, maintenance, and proper fit. A step by step procedure should be outlined and only personnel trained in the disinfection procedure and the respirator program would be allowed to use formaldehyde. Access to the area during the disinfection procedure should be restricted. Detector tubes should be used to determine when it is safe to enter the disinfected area. These tubes can also be used to assure that formaldehyde fumes are not escaping to other laboratories, offices, or public areas.

Maintenance of the fermentation systems, or in the laboratories, should take place only after lock out procedures have been implemented, a verification of clean systems has been made, and clearance from the operator and a supervisor has been obtained.

DIAGRAM 1

FUSION PROTEIN PROCESS - GENENTECH INC. APRIL 1980


Chemical Killing Agent
Steam; Sterilization, Killing Agent
Nutrients
Inoculum
Sample to Verify Inactivation
Batch Fermentation
$10^8 - 10^{10}$ cell/ml.
2 Step Fermentation Cycle Optionable
Cell Harvest via Centrifugation
Fermentation Broth Flushed to Drain
Cell lysis
Centrifugation
Supernatant to drain
Fusion Protein Purification
Enzymatic Cleavage of Fusion Protein
Product Purification via Chromatography

* Currently using 10 liter fermenter, preparing to step up to 600 liter fermenter

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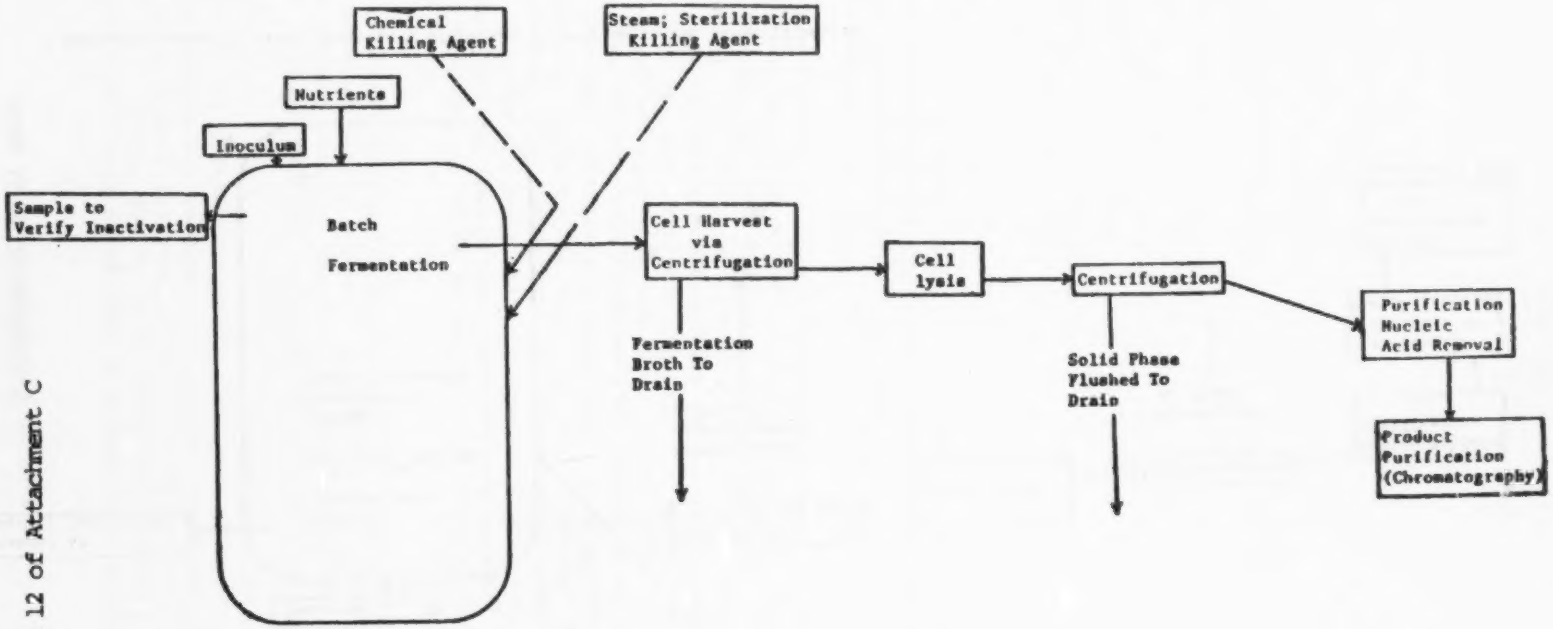
DIAGRAM 2

DIRECT EXPRESSION PROCESS - GENENTECH INC. APRIL 1980


Chemical Killing Agent
Steam; Sterilization Killing Agent
Nutrients
Inoculum
Sample to Verify Inactivation
Batch Fermentation
Cell Harvest via Centrifugation
Fermentation Broth To Drain
Cell lysis
Centrifugation
Solid Phase Flushed To Drain
Purification Nucleic Acid Removal
Product Purification (Chromatography)

Walk-Through Survey Report
Cetus Corporation
Berkeley, California

SURVEY DATE:
April 9, 1980

REPORT WRITTEN BY:
Larry Elliott

SURVEY TEAM:
George Carson
Larry Elliott
Seth Pauker
Dave West

DATE OR REPORT:
September 17, 1980

REPORT NUMBER:
129.11

Industrial Hygiene Section
Industry-wide Studies Branch
Division of Surveillance, Hazard Evaluations and Field Studies
National Institute for Occupational Safety and Health
Cincinnati, Ohio

PLACE VISITED:	**Cetus** Corporation **Berkeley**, California
DATE OF VISIT:	**April** 9, 1980
PERSONS CONDUCTING SURVEY:	**George** Carson **Larry** Elliott **Seth** Pauker **David** West
EMPLOYEE REPRESENTATIVE CONTACTED:	**Ronald** Cape, **Director** of Cetus **(415)** 549-3300
UNION:	**None**
STANDARD INDUSTRIAL CLASSIFICATION OF PLANT:	**7391** Research and Development Laboratories
PURPOSE OF SURVEY:	**To investigate** and evaluate Cetus' **methods** of containment, environmental **monitoring**, and health surveillance **for** production/research processes **utilizing** recombinant DNA techniques.

ABSTRACT

A walk-through industrial hygiene survey was conducted at the Cetus Corporation research laboratories in Berkeley and Emeryville, California on April 9, 1980. This company is involved in research and development of recombinant DNA fermentation processes applicable to chemical synthesis, pharmaceutical preparation, and fuel production. Assessments of control technology, including containment design, validation procedures, and work practices were made during the visit. Recommendations were made to strengthen specific areas in order to minimize potential hazards to the worker.

INTRODUCTION

The public debate over genetic engineering has focused on the possible hazards of gentically modified microorganisms, potential health hazards to workers involved with industrial application of recombinant DNA techniques, and the utilitarian prospects of such technology. Several risk assessment programs designed to investigate some of the characteristics of proposed host-vector systems which might effect hazard potential have been conducted by interested scientists. Likewise, the benefits from recombinant DNA technology are being as vigorously promoted. The National Institute for Occupational Safety and Health (NIOSH) is responsible for conducting research, recommending occupational safety and health standards, providing technical assistance to workers and employers, and conducting training and educational programs. NIOSH's responsibility extends to both existing and emerging technologies which might impact on worker health and safety. Thus, NIOSH is evaluating the potential occupational hazards involved with recombinant DNA technology.

This research effort was prompted by the anticipated surge of recombinant DNA techniques in various industrial processes. Genetic engineering technology may be utilized in various manufacturing processes in the areas of agriculture, organic chemicals, energy, food processing, and pharmaceuticals. This potential growth and the possibility of uncharacterized occupational exposures indicate the necessity for careful evaluation of health risks. NIOSH is accustomed to examining new technologies for potential occupational hazards and developing recommendations for safeguarding the worker's health. Implementation of safeguards and protective engineering controls early in the growth of an industry can minimize human suffering and avoid expensive retrofitting of production systems.

Under the Occupational Safety and Health Act of 1970, Public Law 91-596, NIOSH was mandated and authorized to conduct research and health studies Specifically, section 20(a)7 states that NIOSH shall conduct and publish industry-wide studies of the effects of chronic or low level exposure to industrial materials, processes, and stresses on the potential for illness, disease, or loss of functional capacity. Thus, this research is pursuant to the development of health standards applicable to a broad range of occupational environments. In compliance with this mandate, the Industry-wide Studies Branch of the Division of Surveillance, Hazard Evaluations and Field Studies is conducting a research study to assess the potential occupational hazards in research and the commercial application of recombinant DNA technology.

In order to analyze the current technology for potential occupational hazards, NIOSH plans to conduct several walk-through surveys of recombinant DNA processes and associated industries. Identification of potential hazards, with recommendations to minimize worker exposure, will be derived from the information gained during these surveys.

This report contains recommendations relevant to operations at Cetus, Inc. Recommendations applicable to industries employing recombinant DNA technology will be made after NIOSH completes a thorough assessment. An assessment of the following areas will be made:

- process operations with attendant potentials for worker exposure
- engineering controls (e.g., physical containment design, ventilation, exhaust gas filtration, waste product controls, etc.)
- validation procedures (e.g., of sterilization, physical containment, and process termination)
- work practices
- emergency and accident procedures
- medical surveillance
- environmental monitoring
- numbers of exposed workers
- employee training and education

This survey of Cetus, Inc. in Berkeley, California was conducted as one of a series of initial walk-through surveys of firms utilizing recombinant DNA techniques in research or production.

DESCRIPTION OF THE COMPANY

Cetus started in 1971 as an independent research organization. Cetus conducts research in genetic engineering, biochemistry, and molecular biology. Results of their research may then be developed, on a pilot plant scale, as technology adaptable for commercial application. Cetus plans to market this technology rather than manufacture the products the technology will produce. Research projects are generated from within the company or in collaboration with other organizations.

Cetus is currently conducting research in eighteen project areas. Approximately one-third of these projects involve recombinant DNA research and the remaining projects have the potential for recombinant DNA applications. Pharmaceutical research efforts include the application of recombinant DNA technology for the production of insulin and interferon. The largest part of Cetus' research efforts are centered around organic chemical substitute fuel production. Resulting products of such research would be ethylene and propylene glycol and their oxides, ethanol, alkenes, and substitutes for lubricants and whale oil. Vaccines for animals and humans are also being developed.

The Cetus Corporation is composed of five operating divisions. They are New Programs Development, Biochemical Research Development, Recombinant DNA Research-Group, Fermentation Process Development, and Mutation Screening and Strain Selection Research. These groups have overlapping responsibilities and a project may receive the attention of each division.

DESCRIPTION OF THE WORKFORCE

Cetus currently employs approximately 230 persons of which about 200 are involved with research and process development. Approximately 75% of this workforce have at least bachelors degrees and 50 individuals have Ph.D.'s. Because of the nature of the work, the typical employee hired by Cetus will be highly educated. The pilot scale setting will require very few, if any, line operators.

No structured work schedule exists for employees; most work is conducted during flexible daytime hours.

DESCRIPTION OF THE PROCESS

Process development for the production of insulin, interferon, ethanol, and ethylene glycol is now underway. Development of fermentation, extraction, and purification techniques are also being conducted. Production processes utilizing recombinant DNA will vary depending on the nature of the product desired and the steps required to achieve the product. General process descriptions for specific products were not yet available partly due to the status of the projects.

Cetus's fermentation processes are conducted in 10 liter or less capacities. Fermentation capacities larger than 10 liters will not likely be employed in process research or pilot scale development and therefore, under the current voluntary system, Cetus will not need to submit their project plans to the National Institute of Health Recombinant DNA Advisory Committee for review. Cetus plans to stay at the lab scale or pilot scale level for their research projects.

The directors of the company will not approve risk assessment experiments for in-house project benefit until NIH outlines criteria for such experiments. The company feels that risk assessments, now by the private sector, could be deemed self-serving and a compromise to research.

DESCRIPTION OF INDUSTRIAL HYGIENE, SAFETY AND MEDICAL PROGRAMS

Industrial Hygiene

This company has no industrial hygienist on staff. Environmental monitoring has not been conducted in the laboratories or research areas.

Safety and Health

The company has a formal safety program that consists of written procedures for good laboratory practices. The Safety and Operations Manual for the Cetus Corporation P-3 facility is contained in Appendix 1.

An Environmental Health and Safety Manager directs the safety program. He insures that individual employees receive training in good work practices and periodic safety checks are made during work periods. Laboratory coats, safety glasses, safety shoes, and respirators (where required) are provided by the company. Clothes changing areas with showers and lockers are also provided.

Several employees are qualified to provide first aid and cardiopulmonary resuscitation (CPR). There is no licensed nurse at this facility. A health clinic, which can provide emergency medical care, is located two blocks from the Cetus research building.

An Institutional Bio-Safety Committee (IBC) has been formed in compliance with the NIH guidelines. The IBC is composed of nine members; two of which are outside consultants. Meetings are held when needed to approve Memorandum of Understanding and Agreement (MUA) for NIH, discuss changes in NIH guidelines, or for modification in research projects.

Medical Programs

Pre-employment physicals or comprehensive annual medical examinations of employees are not required by Cetus. All injuries must be reported to the employee's immediate supervisor. Employees on sick leave must have clearance from a physician before returning to work. Employees with open wounds, or those on antibiotic therapy, are excluded from working in recombinant DNA areas.

Pre-assignment and annual blood serum samples are maintained for employees working with recombinant DNA techniques. The IBC is investigating the need for specific medical tests or medical certification prior to working in recombinant DNA areas.

DESCRIPTION OF PAST EXPOSURES AND POTENTIAL HEALTH HAZARDS

There is no exposure data available because environmental monitoring is not conducted. Thus, no qualitative or quantitative results exist to document range or incidence of exposure to research material.

Other potential health hazards are radioisotopes, and organic chemicals, such as, chloroform, formic acid, toluene and formaldehyde. Proper usage, storage, and disposal requirements have been discussed by the company with the employees involved with these hazards.

EVALUATION OF THE PLANT AND PROGRAMS

Physical Containment Design

Several P-2 and P-1 containment level laboratories and a very well designed P-3 laboratory were inspected. The design and operation of these laboratories were well within the NIH guidelines. Berkeley and Emeryville each have municipal ordinances that require biological, genetic, and molecular biological research be conducted within guidelines similar to, and in some regards more stringent than, the NIH requirements. In essence, by complying with the Berkeley and Emeryville ordinances, Cetus is operating within the NIH guidelines.

Engineering Controls

The ventilation systems, exhaust gas filtration, waste products control, biological safety cabinets, access barriers, and all interior room surfaces in the P-3 and P-2 laboratories were within the NIH guidelines. All laboratories were well planned, constructed, and utilized. With minor equipment changes, the P-3 level laboratory could be used as a P-4 laboratory.

All liquid wastes from the P-3 laboratory are treated in a bio-waste system located on the plant site.

An automated security computer system monitors the ventilation systems, access areas, safety cabinets, centrifuges, autoclaves, and ambient environment of the laboratories. The performance of this system is checked periodically by the company which installed it.

Validation Procedures

All biological safety cabinets and walk in hoods receive scheduled performance testing and certification. Autoclaves are monitored by integral recording charts to document performance.

All centrifuge equipment receives periodic maintenance checks. Validation of active sterilization is conducted on systems required under the NIH guidelines.

All of the above mentioned procedures are in addition to the continuous monitoring by the security computer system.

Work Practices

Specific guidelines for safe work practices and laboratory techniques have been promulgated by the Environmental Health and Safety Manager and the company safety committee. Employee observance of these guidelines seemed very good.* There was no evidence of drinking, eating or smoking in the laboratories and all the workers were wearing the appropriate protective clothing.

Maintenance of equipment, of systems, or in the laboratories takes place only after clearance from a supervisor has been obtained.

Personnel working in the P-3 laboratory are responsible for the cleanliness of the area. Personnel in other laboratory areas are also responsible for the general cleanliness of their respective laboratory, but a janitorial service cleans the floors and removes the trash.

Detailed requirements for work practices in the P-3 laboratory can be found in Appendix 1.

Emergency and Accident Procedures

Safety precautions and plans for emergencies, such as laboratory accidents, are outlined for each laboratory containment level and research application.

CONCLUSIONS

General experience has demonstrated that safe conduct of research, or production processes, involving hazardous entities is dependent on good work practices, availability and use of effective control technology, and effective management. Microbiological research requires containment features sufficient enough not only to exclude entry of contaminants into, but also prohibit escape of organisms from the system. Thus, attention to exposure control must receive equal emphasis as process integrity and production development. Safety and health, medical, and emergency programs need to be implemented and managed with the same degree of attention that the scientific production programs receive. It is evident that Cetus recognized the importance of safety and health programs and implemented these early in the corporate development. Cetus is conducting its research and development of recombinant DNA in a well-conceived and adequately designed work environment.

RECOMMENDATIONS

The medical program should consist of pre-employment and annual physical examinations in order that employees may be monitored for abnormal health effects. Immunizations should be considered for employees working with known pathogens. Employee health records should be maintained and include notes on all illness, operations, physical disorders, therapeutic medications, and accidental exposures.

A registry of all workers employed with recombinant DNA techniques should be established and maintained for future reference to determine any abnormal medical problems, or unusual occurances, that might be attributable to occupation. NIOSH has had considerable experience in designing and maintaining such worker registries and can, thus, provide assistance in design, implementation, and logistical requirements. The comparability of individual company registries is desirable for optimum and successful surveillance on an industrial wide basis.

Biological monitoring of worker body fluids for specific organisms carrying recombinant DNA molecules is costly and technically demanding. Quantitative and qualitative sampling of air and surfaces in the production and lab areas can be an effective measure of sanitation, environmental conditions, and efficacy of control measures. Environmental sampling programs have been utilized in hospitals, the food industry, and containment laboratories for years and the principles of these programs are applicable to the operations at Cetus. The efficacy, sensitivity, and accuracy of environmental sampling and analytical techniques should, however, be considered and their validity must be established prior to utilization. The procedures, analyses, sampling points, and sampling schedules, thus established, should be formalized in writing.

The use of formaldehyde as a disinfectant agent in laboratory areas merits certain precautions. Employees should wear rubber gloves when working with formaldehyde and when contact with the hands is likely. If the disinfection process involves occasional brief exposures to formaldehyde at concentrations in excess of 1.2 mg/cubic meter, an appropriate respirator should be used. A respirator training program would then also be required (Cetus does have one in effect), with emphasis on cleaning, maintenance, and proper fit. A step by step procedure should be outlined and only personnel trained in the disinfection procedure and the respirator program would be allowed to use formaldehyde. Access to the area during the disinfection procedure should be restricted. Detector tubes should be used to determine when it is safe to enter the decontaminated area. These tubes can also be used to assure that formaldehyde vapors are not escaping to other laboratories, offices, or public areas. If it is determined that use of formaldehyde can not be adequately controlled,. a substitute agent should be utilized.

The use of the P-3 level laboratory for P-1 or P-2 level work was mentioned by the Cetus representatives. If the P-3 area is used consistantly for work allowing relaxed procedures, as compared to P-3 level requirements, the Safety and Operations Manual for the P-3 Facility should be revised. Additional practices, procedures, and safety precautions should be outlined for such use of the P-3 facility. The revised policy, and inherent concerns, should then be discussed with all personnel involved and then closely supervised to assure the policy is adhered to.

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